REMARKS

The enablement rejection under 35 USC §112, first paragraph

The examiner maintained the previous rejection of claims 44, 81 through 90, 92 through 98, 102 through 107 and 123 under 35 USC §112, first paragraph, for allegedly lacking enablement in the specification for reasons previously set forth. The examiner acknowledged that upon reconsideration, Exhibit 3 previously made of record includes data that support a trend toward regression of CNV, but asserted that in Exhibit 5, also of record, the dosage falls outside the dosage range supported by the specification. Regarding dosages, the examiner acknowledged applicants' position that the 50 mg/kg dose in mice of Exhibit 5 roughly corresponds to a 4.2 mg/kg dose in human and applicants' reliance on the disclosure of Freireich to support this position. In rebuttal however, the examiner asserted that "this reference is drawn to the determination of the maximum tolerated dose in man based on the approximate LD10 in various animals" and that "[t]here is nothing in this reference to support the argument that the same conversion factor of 1/12 is accepted in the art for determining efficacy."

Addressing first the examiner's statements regarding the disclosure of Freireich, the examiner's comment is correct; the referenced disclosure does address conversion factors for maximum tolerable dosage in man based on determinations made in various other animals. The values are, as the examiner states, expressed in terms of lethal dosage, and the lethal dose for a mouse is stated as being $1/12^{th}$ that which would be predicted for human. The applicants submit that effectiveness for producing a lethal result is, while admittedly extreme, a measure of drug efficacy, and the examiner has offered no evidence to dispute such a position. Moreover, the examiner has not offered any evidence that disputes the position taken in the cited reference that the $1/12^{th}$ conversion factor is not accepted in the art.

Furthermore, persons skilled in the art at the time of the September 4, 1998 filing of the priority application for the subject application would have known that the extrapolation factors from animals to humans of maximum tolerated doses of cancer chemotherapy drugs from toxicity studies would also be useful factors in the extrapolation of therapeutic doses from animals to humans, because the effective doses of these drugs are often near or above the toxic doses.

> The application of the surface area rule to drug dosage calculation clearly involves toxicity as an important consideration. Cancer chemotherapy focused attention on the extrapolation of toxic effects because these drugs are often effective only at levels near or above toxic levels. As a result, the extrapolation from animal studies to human treatment is critical.

Chappell et al., 1991, "Extrapolation of toxicology and pharmacological data for animals to humans." Adv Drug Res, 20:1-116 at page 24 (a copy of which is attached hereto as Exhibit A).

The applicants note that the Freireich reference is cited in this 1991 reference (Exhibit A) as the classic study regarding obtaining accurate quantitative methods for extrapolating the results of anticancer drugs from animals to humans (*Id.* at page 25, 4th paragraph).

As discussed below in applicants' remarks regarding the written description rejection, the thiomolybdate compounds of the subject invention are useful for treating diseases of aberrant vascularization including both ocular neovascularization and malignant tumors having a vascular component. Since one skilled in the art would have known to extrapolate dosage information from animals to humans in the context of cancer chemotherapy, the skilled artisan would also have known that such an extrapolation was possible for doses regarding a disease with the same mechanism of achieving therapeutic effect, i.e. ocular neovascularization.

Furthermore, the conversion factors taught by Freireich continue to be used by skilled artisans as demonstrated by the current FDA guidance for estimating dosages for clinical trials. "Guidance for Industry and Reviewers – Estimating the Maximum Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers" published by the US Department Of Health and Human Services, the Food and Drug Administration, the Center for Drug Evaluation and Research which published in July 2005 (a copy of which is attached hereto as Exhibit B). The examiner's attention is directed to Table 1 at page 7 which shows that to convert animal dose in mg/kg to human equivalent dose (HED) in mg/kg, one either divides the animal dose by 12.3 or multiplies the animal dose by 0.08.

Accordingly, the 1/12th factor for converting mouse dosages to human dosages is at least accepted by the US government. If the examiner has any evidence that

demonstrates to the contrary that such a conversion factor is not accepted in the art, the applicants respectfully request that such evidence be provided. Otherwise, the rejection for purported lack of enablement must be withdrawn.

The written description rejection under 35 USC §112, first paragraph

The examiner also rejected claims 44, 81 through 90, 92 through 98, 102 through 107 and 123 under 35 USC §112, first paragraph, for assertedly lacking written description in the specification. The examiner acknowledged applicants' position that 3.0 mg/kg or so translates into a dosage of about 210 or so for a 70 kg human and that the specification teaches daily dosages as high as 410 mg/daily in Wilson's disease patients. The examiner's concern was whether a dosage for treating Wilson's disease is descriptive of dosages for the recited methods, and even if it were, the examiner asserted this disclosure does not describe a range greater than 200 mg/kg. Without any admission that the examiner's position is correct, the applicants offer the following.

Applicants note that each case requires an analysis of the facts to determine "whether an application conveys to those skilled in the art the information that the applicant invented the subject matter of the claims." *In re* Wertheim, 541 F.2d 257, 263 (CCPA, 1976).

The specification provides, at paragraph [0168] of U.S. Patent Application Publication No. US 2005-0058720 A1, that compositions and methods of this invention are "... applicable to the treatment of any malignant tumor having a vascular component." The subject application also provides guidance for selecting appropriate doses of the compositions of the subject invention at paragraphs [0175]-[0176]. Specifically, in paragraph [0178], the subject application describes "Thus, in particular aspects of the invention, loading dosages of ... greater than 200 mg...are contemplated by the inventors as exemplary daily loading dosages..." Finally, the specification at paragraph [0181] also teaches that ocular neovascularization, like cancer and solid tumors, is a disease state characterized by aberrant vascularization and which can be treated or prevented by the thiomolybdate compositions of the subject application.

Since the subject application discloses the treatment of malignant tumors with aberrant vascularization with a loading dosage of greater than 200 mg of a thiomolybdate

compound and the specification also discloses that thiomolybdate compounds can be used to treat other diseases characterized by aberrant vascularization such as ocular neovascularization, it would be clear to one skilled in the art that a disease characterized by ocular neovascularization could be treated by a loading dosage of greater than 200 mg of a thiomolybdate compound as recited in claim 44, since the mechanism of achieving therapeutic effect is the same.

The applicants therefore submit this disclosure discussed above standing alone or viewed in combination with the previously submitted response effectively demonstrates that the written description requirement of section 112 is satisfied and the rejection must be withdrawn.

The double-patenting rejection

The applicants again acknowledge the rejection of claims 44, 83 through 85, 104, 106, 107, and 116 through 122 over claims 43 through 48 and 57 in US Patent No. 6,703,050 and submit that, upon notification from the examiner that the instant claims are in condition for allowance, a terminal disclaimer will be duly filed.

Dated: August 21, 2007

Respectfully submitted,

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CONTENTS

Extrapolation of Toxicological and Pharmacological Data from Animals to Humans

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LIST OF ABBREVIATIONS

AUC	Area under the curve
BME	Body mass equivalence
BMR	Basal metabolic rate
CL	Clearance
DCM	Dichloromethane
ECW	Extracellular water
EDC	Ethylene dichloride
EPA	(US) Environment Protection Agency
GSH	Glutathione
GST	Glutathione-*S*-transferase
ICW	Intracellular water
LOAEL	Lowest observed adverse effect level
MFO	Mixed-function oxidases
MTD	Maximum tolerated dose
NOAEL	No observed adverse effect level
PB-PK	Physiologically-based, pharmacokinetic (model)
RME	Residual mass exponent
SAE	Surface area equivalence
TBW	Total body water

1 Overview

In this chapter we review the literature concerning the extrapolation of toxicological and pharmacological data from laboratory animals to humans and draw conclusions regarding such extrapolations. Four extrapolation models are discussed.

- *Body mass equivalence* (BME), whereby it is assumed that the equivalent dose in milligrams or milligrams per day is proportional to body mass.
- *Surface area equivalence* (SAE), whereby it is assumed that the equivalent dose is proportional to body surface area.
- *Allometric models*, whereby it is assumed that the relevant measure of toxicity (e.g. the LD_{50} or the lowest observed adverse effect level, LOAEL) is a power function of mass, with empirically determined coefficients and exponents.

- *Pharmacokinetic models*, whereby pharmacokinetic models are used to simulate the fundamental processes governing the absorption, distribution, metabolism and excretion of chemicals in the body.

This chapter discusses these models in terms of their historical development, empirical bases, pharmacological applications and use in toxicological extrapolation. The discussion is entirely based on information existing in the literature. On the basis of this review we make the following conclusions.

(a) *Regarding the surface area and body mass models*

- The body surface area model is best viewed as a surrogate for complex and incompletely understood mechanisms.
- In view of the difficulty of measurement of body surface area, combined with the inevitable uncertainties in other measurements, there is no reason to use complex equations for surface area calculations. It is quite adequate to assume that

$$\text{surface area} = cM^l,$$

  where M is body mass, with the same value of c and l for all species. Typically the value of l encountered in the literature is 2/3; however, in view of the many uncertainties in the data, the exponent l could as easily be 0.7 or 0.75.
- SAE will always predict a smaller dose in milligrams or milligrams per day for humans than BME when extrapolating from smaller animals to humans. Furthermore, while there is a great lack of uniformity in the toxicological literature, the data tend to support SAE over BME.
- The physiological support, both theoretical and empirical, for SAE is equivocal; in spite of many attempts, no convincing proof of the SAE model exists, however the empirical evidence leans more in favour of SAE than BME.
- Where no data other than toxicological data on one or two laboratory animals is present, the most conservative approach (in terms of minimizing false-negatives) and the approach best supported by existing evidence is to use SAE rather than BME to extrapolate to humans.

(b) *Regarding the allometric models*

- The "surface law" (SAE) and the "body weight law" (BME) should be considered subsets of the allometric model where the dependent toxicological parameter Y (e.g., maximum tolerated dose) depends on body mass (M) according to

$$Y = bM^k.$$

Both b and k are determined empirically and are assumed to be the same for all species. In the case of BME, the value of k is 1 and in the case of SAE, it is 2/3. In the early 1930s, another popular model came into existence. This model, often known as the Kleiber–Brody Law, states that basal metabolism rates scale with an exponent of 0.75 rather than 2/3 (which is equivalent to SAE). This model was first developed by the application of linear regression analysis to metabolic data for many species. There have been some interesting theoretical arguments put forward to attempt to provide a general proof for the value of 0.75 for the allometric exponent. While many of these arguments are appealing, they do not offer an unequivocal proof that the exponent is 0.75.

- The existence of such allometric relationships for a wide variety of physiological parameters is well-documented. These relationships are probably a reflection of fundamental physical, chemical and biological constraints on natural selection.
- The vast body of empirical data (including data used to support SAE) supporting allometric relationships of a wide variety of fundamental physiological parameters strongly suggests that it is possible, in many cases, to extrapolate pharmacological and toxicological data from animals to humans.
- The application of allometry to toxicological and pharmacological dose extrapolation frequently results in values of k between 0.6 and 0.8. These values are not very different from 0.67 for the surface area law or 0.75 for the Kleiber–Brody Law, but generally they are significantly different (both in a statistical sense and in their consequences) from the value of 1 for the BME model. In view of the uncertainties, the value 0.7 for the exponent seems a reasonable compromise that is not inconsistent with the surface law (SAE) or the Kleiber–Brody law (the use of 0.7 to extrapolate from a mouse to a human gives a result differing by roughly 30% from that obtained using either 0.75 or 0.67). Indeed, one danger in using two significant figures is that it can give the appearance of greater certainty than actually exists.
- In the context of the allometric model, where mass is a surrogate rather than a cause, it is more appropriate to view k simply as an empirical parameter. Preferably the exponent should be empirically determined. If, however, extrapolation must be made on the basis of inadequate data, we recommend the use of the allometric equation with an exponent of 0.7. The result will be more conservative than the use of BME (exponent = 1) and, in view of the many uncertainties in measurement, essentially indistinguishable from the result obtained using either the surface law (SAE) or the Kleiber–Brody law.

(c) *Regarding the pharmacokinetic models*

- Developments in pharmacokinetics over the last two to three decades lend further support to the idea that toxicological and pharmacological data can often be extrapolated from laboratory animals to humans.
- Pharmacokinetic concepts, such as clearance and distribution volume coupled with the allometric model, provide a more satisfactory mechanistic basis for extrapolation than surface area equivalence or the Kleiber–Brody law for extrapolating pharmaceutical and toxicological data.
- Although the value of k is frequently in the range of 0.6–0.8, as mentioned above, there are chemicals where the exponent is neither unity nor in the range 0.6–0.8; therefore no single exponent can be used in all cases, and the safest approach is to empirically determine the best value for k in the particular case of interest.
- The so-called allometric pharmacokinetic model, where allometric equations are used to scale pharmacokinetic parameters such as volume of distribution, half-life and clearance, is probably adequate for a large number of chemicals. Because of the empirical and "black box" nature of this model, it is not clear exactly when this approach is inadequate and when it needs to be replaced by more complex approaches. Additional research is needed to clarify this issue.
- The physiologically-based, pharmacokinetic (PB-PK) model, which involves detailed mass-balance calculations for organs and tissues believed to be important in a compound's disposition, is viewed by some workers (e.g. Anderson *et al.*, 1987) to have a greater potential than the allometric pharmacokinetic model in providing accurate predictions; however, others disagree with this assessment. These models are very labour-intensive and costly and have only been used for a limited number of chemicals.
- It is possible, for some chemicals, to use short-term, relatively inexpensive experiments to develop the parameters needed for toxicological and pharmacological extrapolations.
- In view of the complexity of the PB-PK models, the allometric pharmacokinetic model is probably adequate and more practical, except where it can be demonstrated that it will not work.
- In view of physiological differences among humans, including effects of age, disease and gender on clearance and other pharmacokinetic parameters, it is prudent to use a safety factor when extrapolating doses across species, even if it is felt that the pharmacokinetic model in a particular case is quite accurate.
- The pharmacokinetic and toxicological data base is inadequate to make valid comparisons of the different extrapolation models discussed here.

We believe that the use of allometry coupled with pharmacokinetic data can not only provide for more accurate extrapolation, but can also significantly reduce the cost involved in determining reference doses and other parameters required for setting environmental standards or determining Phase I doses for therapeutic agents. However, there is a need to improve the knowledge base in this area.

It is clear that the paucity of data does not allow for much confidence in extrapolation from animals to humans. In view of the importance to public health and the possible economic impacts of such extrapolations, it is important to improve our knowledge in this area. In the case of toxic chemicals released into the environment, an incorrect value could have public health impacts (by being too high) or economic impacts (by being too low) that could easily "pay" for the research needed to avoid such mistakes. Since the economic cost of inaccurate extrapolations is borne by government, industry and the general public, it is entirely appropriate that the cost of such research should be supported by government and industry. Clearly, this research has applications not only to the toxicology of environmental contaminants, but also to drug testing and the selection of treatment protocols (e.g. chemotherapy). On the basis of our review we recommend the following.

(1) Research should be carried out to develop the data required to test various extrapolation models for a number of chemicals representing different classes of chemical and toxicological profiles. These studies should include area under the total and unbound blood concentration versus time curve for both single- and multiple-dose experiments.
(2) A protocol should be developed that would involve acute and chronic toxicity studies on perhaps two species, with short-term pharmacokinetic studies on four to five species. The latter studies would be used to develop allometric equations, for clearance, volume of distribution and half-life, that could then be used to extrapolate the results of the toxicity studies. Numerous factors can affect pharmacokinetic variables such as clearance; these include age, genetic variability, sex, illness and chemical exposures. The determination of appropriate safety factors can be addressed by research programmes, but is ultimately a policy decision.

2 Introduction

Laboratory animals have often served as models for the study of humans. This use has been based on the assumption that the extrapolation of biological data from such animals to humans is valid, at least for some physiological parameters. In recent years, the perceived need to develop regulations for chemicals in the environment, as well as the development of synthetic drugs, has greatly increased the use of animal models for toxicological and pharmacological extrapolation.

Three extrapolation techniques are used by regulatory agencies. One of these is the use of body mass or what we will call body mass equivalence (BME). It assumes that the equivalent dose (i.e. the dose in milligrams or milligrams per day, depending on whether it is a single- or multiple-dose situation) is proportional to body mass. Another way to state this is that the same dose as milligrams per kilogram of body mass or milligrams per kilogram of body mass per day will have the same effect in all species.

Another widely used approach has been the use of body surface area. In this approach, which we will call surface area equivalence (SAE) (more widely known as the surface law or surface area rule), it is assumed that the equivalent dose in mg or mg/day is proportional to the body surface area of the animal. Thus, if the same chemical is given to animals in such a way that the dose per surface area in milligrams per square metre or milligrams per square metre per day is the same for all the animals, then the response to the chemical will be the same.

A third approach is given by assuming that, for chronic experiments, equal proportions of the diet will lead to similar effects. That is, if a chemical is given to an animal as 10 ppm in the diet and has a particular effect, it will have the same effect on all other animals if given as 10 ppm in their diets. This method generally gives results similar to those given by the "surface rule" described below.

The assumption of BME is very natural and was probably the earliest method used. Indeed, it would have been very natural to assume that the equivalent dose of a drug for a 5 kg child is about 1/15th of that for a 70 kg adult; however, as early as 1830 it was recognized that this approach sometimes led to poor estimates. When an adult was given 15 times the dose safe and effective for children, the adult would sometimes suffer toxic effects. Conversely, when a child was given 1/15th of the safe and effective dose for adults, the result often was an inadequate pharmacologic response. Interestingly, at about the same time as this problem was first reported (1830), two French investigators (Sarrus and Rameaux, 1838) proposed that energy metabolism in animals is proportional to their surface area and that the surface area of an animal is proportional to the two-thirds power of its mass (which explains why the method involving equal proportions of the diet gives similar results). It was not until the first decade of this century that a connection between energy metabolism, surface area and toxicity was made.

Since 1910, a number of investigators have considered the general problem of intraspecies and interspecies scaling or extrapolation. Much of this work has focussed on issues other than, but potentially related to, toxicity. That is, investigators have considered the scaling of the size of organs (heart, liver, kidney, etc.), the function of organs (heartbeat, breath rates, enzymatic activity, etc.) and parameters related to drug metabolism (half-life, clearance, distribution volumes, etc.). A considerable literature has developed regarding the relative success of the attempts to scale these and other physiological parameters. While the vast majority of these investigations did not directly relate to extrapolating toxicity data, clearly the various physiological parameters mentioned above have an important bearing on the toxic effects of chemicals. Thus, those techniques that have been successful in extrapolating relevant physiological parameters, such as the rate at which a chemical is cleared from the blood, should have a reasonable chance of working for toxicological extrapolations. Indeed, comparisons involving toxic endpoints have been made using chemotherapeutic agents. Surface area equivalence was reasonably successful in predicting the toxic effects (Freireich *et al.*, 1966) of many such agents. This approach has also been successful in predicting the therapeutic level for a variety of other drugs (Crawford *et al.*, 1950).

Nearly 100 years after Sarrus and Rameaux (1838) proposed that energy metabolism rates (generally meaning basal rates) for animals are proportional to the two-thirds power of the mass, Kleiber (1932) and Brody and Proctor (1932) reported that the use of linear regression analysis of the metabolic data gave the result that energy metabolism was proportional to the three-fourths power of the mass. The use of linear regression analysis on data for organ weights and other physiological parameters led to numerous equations of the type

$$Y = bM^k, \tag{1}$$

where M is the body mass, Y is the physiological parameter of interest (e.g. liver weight), and b and k are constants. These equations were obtained by fitting the equation to both intraspecies and interspecies data. In a sense, this approach contains the surface area approach as a special case ($k = 2/3$). But in another sense, the approach is different in spirit because it uses body mass or a power of body mass as a surrogate and is strictly empirical in nature.

This approach is called allometry or the study of size. Haldane (1928) has noted that "the most obvious differences between different animals are differences of size, but for some reason the zoologists have paid singularly little attention to them". Galileo may have been the first allometrist. He pointed out that the effect of gravity has serious consequences for the

structure of animals (Galileo, 1637). This can be seen by taking an example from Haldane (1928). Suppose we considered a giant man 60 ft high, and we scaled everything linearly so that all dimensions increased by ten. Then these monsters would weigh a thousand times as much as a 6-ft man. But the cross-sections of their bones would only be one hundred times those of a normal sized person; as a result the breaking strengths of the bones would be exceeded. The only way out of this dilemma is to change the structural material, allow the diameters of the supporting limbs to increase at a faster rate (to give an appearance like a rhinoceros or elephant), or to compress the body and stretch out the legs obliquely to gain stability (like a giraffe).

There are numerous consequences of size, including the need to change shape as described above, as well as the existence of minimum (the shrew) and maximum (the elephant) sizes for land mammals. The study of these consequences is a part of allometry. One such consequence of size is the way an animal responds to a toxic agent. It is often noted that a small animal can sometimes tolerate a larger quantity in milligrams of the agent per kilogram of body weight than a large animal (Freireich *et al.*, 1966). In view of the vast number of physiological parameters which obey allometric equations (Peters, 1983), it is reasonable to expect that a parameter measuring some aspect of response (LD_{50}, LOAEL, etc.) would for some chemicals obey an allometric equation.

The implication made is that fundamental physiological processes govern the response to toxic agents and that these processes are a function of size. The link between the response and the processes involves an understanding of the pharmacokinetics (e.g. absorption, distribution, metabolism and excretion) of the chemical. In the last 20 years, considerable progress has been made in developing models to predict the effects of chemicals on humans from animal data. While much of this work has focussed on pharmacological responses, the same principles apply to toxicological responses.

The fundamental concepts of pharmacokinetics deal with parameters such as plasma concentration, clearance, biological half-lives, enzymatic rates, organ volumes, and other physiological parameters describing the absorption, distribution, metabolism and excretion of a chemical in the animal system. In many cases, these parameters obey allometric equations, thereby providing the link between toxic response and a power function of the mass. Several books and papers have reviewed the various scaling techniques; these include Brody (1945), Kleiber (1961), McMahon and Bonner (1983), Calabrese (1983), Calder (1984), Schmidt-Nielsen (1984), Davidson *et al.* (1986), and Boxenbaum and D'Souza (1990).

In Section 3 we discuss the concept of the surface law SAE. We review the literature on energy metabolism, surface area measurement and formulae,

organ sizes, body fluids, and other physiological parameters. We then discuss the data on the application of SAE to drug metabolism and toxicity.

In Section 4 we discuss allometry, reviewing the literature on differences from the surface area law for energy metabolism, the empirical basis for allometric equations, the theory of biological similarity and the concept of physiological constants. We then discuss the application of allometry to the scaling of toxicity data.

In Section 5 we discuss some of the developments, in the past two or three decades, in pharmacokinetics which employ the scaling issue. We review the literature on the dependence on mass of elimination half-life, clearance, distribution volumes and area under the concentration–time curve. We review the literature on predicting plasma concentration as a function of dose and time, and on methods for scaling of dose and time, which have been very effective in interspecies scaling. Finally, we discuss the application of two pharmacokinetic models, the allometric model and the PB-PK model(s), to toxicological and pharmacological extrapolation.

3 The surface area law

3.1 INTRODUCTION

Similarities in structure and form in animals have been investigated for at least three and a half centuries (see Galileo, 1637, for example). In all probability, the earliest scaling hypothesis was that all physiological parameters were proportional to body mass. In this section, we review the historical development of the surface area law, describe some of the ways to measure surface area, discuss some of the mathematical formulae proposed for calculating surface area and review the literature regarding application of the surface area law to drug dosage and toxicity. The surface law has drawn the attention of a number of investigators. Some of these were interested in establishing a basic tenet of biology while others treated it less as a law than as a surrogate for a more fundamental parameter. We are interested in its application to scaling data on chemicals from laboratory animals to humans.

In 1838, Sarrus and Rameaux developed the surface law for the energy metabolism rates of homeotherms. Since energy loss is proportional to the surface area of the body (Fourier's law), they reasoned that energy must be produced at a rate proportional to surface area of the animal, in order to maintain a constant internal temperature. This proportionality between energy metabolism and surface area is the "surface law". In fact, the proportionality constant in Fourier's law is not a constant, but depends on

the characteristics of the body (Lee and Sears, 1963). Thus, while true on a differential ("infinitely small") scale, it is true on a macroscopic scale only for strictly homogeneous bodies. As a result, small deviations from an exact proportionality between heat loss and body surface area for a system as complex as an animal are not surprising.

This proposal by Sarrus and Rameaux helped explain the phenomenon of increasing energy metabolism per unit body weight with decreasing size; that is, a 2 kg rabbit has an energy metabolism of about 75 kcal/kg per day whereas a 440 kg horse has an energy metabolism of about 10 kcal/kg per day. (Normally the measurements are made on a fasting and resting animal to obtain the basal metabolic rate which is the minimal rate of heat production.) By assuming that animals have the same density and similar shapes, Sarrus and Rameaux (1938) showed that the surface area of an animal is proportional to body mass to the power two-thirds. When measurement techniques for surface area became available, investigators attempted to check the surface law and obtained results that indicated good agreement. A number of measurement methods were developed, including covering the animal with a cloth, using a roller with a known area and counting revolutions, and skinning. While these measurements were first done for intraspecific comparisons, the application to interspecific comparisons followed very quickly.

As we will show in Section 3.2.3, in order to have the energy metabolism proportional to surface area, other physiological parameters need to scale with mass to a power less than one. Numerous measurements were made of various organs, body fluids and other physiological parameters and compared with surface area. Several investigators reported that organs such as the kidney, liver, thyroid and heart had masses proportional to the body surface area (i.e. the mass exponent is 2/3). Others reported that various fluid volumes, such as whole blood, scaled in the same way. Many of these reports were later found to be incorrect; for example, there is now general agreement that blood volume is proportional to body mass (i.e. the mass exponent is 1) (Prothero, 1980).

One of the most interesting applications of the surface area rule has been to drug dosage. In this context, the dose required, in milligrams or milligrams per day divided by the surface area, for a given response, is a constant from one species to another (interspecies extrapolation) and within species (e.g. from infants to adults). Done (1964) notes that sulphonamides were used to treat infections in infants at doses of 100 mg/kg; in an adult this would lead to a dose of 7 g, which is grossly excessive. Moore (1909) proposed that equivalent doses should scale as the surface area or the two-thirds power of the mass. This idea seemed to go through several cycles of acceptance and rejection, as investigators would report success in its use but

then find it difficult to describe an appropriate mechanism to explain the role of body surface area.

In the early 1950s, the surface area rule was in widespread use in some hospitals (Crawford *et al.*, 1950). It is still widely used in determining the dose of chemotherapeutic drugs.

The introduction of chemotherapeutic drugs led to a considerable interest in extrapolation methods. Pinkel (1958) reported that the generally accepted doses for several of these drugs scaled as the surface area. The use of chemotherapeutic drugs also generated considerable data on human toxicity. In 1966, Freireich *et al.* reported on an extensive review of toxicity data for chemothcrapeutic drugs. They found that the toxic dose for a large number of these drugs scaled approximately as the surface area or the two-thirds power of body mass. These findings have played an important role in determining safe starting doses in human testing (or Phase I trials).

3.2 LITERATURE REVIEW

3.2.1 Energy Metabolism and the Surface Area Law

Sarrus and Rameaux (1838) proposed that there is a regular relationship betwcen heat production and size given by the relationship

$$\text{heat production} \propto \text{surface area}. \tag{2}$$

Their reasoning was based on laws of physics governing the loss of thermal energy by bodies; namely, that the rate of cooling of a body is proportional to its surface area (as mentioned previously, this relationship strictly holds only for bodies with homogeneous thermal properties). For homeotherms, heat loss must equal heat production. They also reasoned that heat production is proportional to oxygen consumption and, thus, roughly proportional to inspired air.

If we assume that two animals have the same shape but that the corresponding linear dimensions of one are X times those of the other, then the surface area and volume of that first animal are X^2 and X^3 times those of the second, respectively. If the two animals have the same density, then the mass (M_1) of the first one is X^3 times the mass (M_2) of the second, i.e.

$$M_1 = X^3 M_2, \tag{3}$$

therefore

$$M_1^{2/3} = X^2 M_2^{2/3}. \tag{4}$$

As a result the two-thirds power of the masses of the two animals (with similar shapes and equal densities) bear the same relationship as their surface areas (X^2). Consequently, the surface area, S, can be related to mass, M, by

$$S = a_1 M^{2/3}, \tag{5}$$

where a_1 is a constant. Thus from eqs (2) and (5), the metabolic rate, P, is given by

$$P = a_2 M^{2/3}, \tag{6}$$

where a_2 is another constant.

Sarrus and Rameaux (1838) restricted their considerations to intraspecific comparisons where similarity of shape is easier to obtain; even with this restriction, however, there are a number of assumptions involved. One is that eq. (2) initially comes from Fourier's law concerning heat flow. This law also involves the heat conductivity of the surface materials. It is well known that even among litter mates, two animals will grow different amounts of fur if subjected to different external temperatures (Kleiber, 1947). Thus, one might argue that instead of natural selection operating to require energy metabolism to be proportional to surface area, it might have operated to give small animals a much greater insulation than large animals in such a way as to make energy metabolism proportional to mass. However, Kleiber (1947) points out that to maintain its body temperature in an environment of 3°C, a 60 g mouse with the same metabolic rate per unit weight as a steer would need the equivalent of a steer's surface covering in a layer 20 cm thick. (Such a mouse would appear as a ball of fur about the size of a volleyball!) This example is a good illustration of why it is advantageous for a small animal to have a higher metabolic rate per unit mass than a large animal.

Kleiber (1947) also reviewed the various mechanistic theories advanced to support the belief that the metabolic rate of animals is proportional to body surface area. The theories are as follows:

(1) The rate of heat transfer between animal and environment is proportional to the body surface area.
(2) The intensity of flow of nutrients, such as oxygen, is a function of the sum of internal surfaces, which is proportional to body surface area.
(3) The rate of supply of oxidizable material and oxygen to the tissues is a function of the mean intensity of the blood current, which is proportional to the area of the blood vessels, which in turn is proportional to the body surface area.
(4) The composition of animals, both anatomically and chemically, is a function of their body size, and larger animals have relatively more structural material and less "metabolically active" mass.

(5) The cells of the body have an inherent oxygen requirement found proportional to body surface area.

Kleiber found only theories 1 and 3 to have merit and proposed that the most attractive theory involves an integration of the heat exchange and circulatory theories. He suggested that "In natural selection, those animals probably prove to be the fittest whose cells are adapted to such a level of oxygen consumption that the metabolic rate of the animal is most suitable for the maintenance of a constant body temperature and in line with the most efficient transport of oxygen." He noted that if a 4 ton animal required the same rate of oxygen consumption as that of a mouse, it could not survive because this metabolic rate could not be sustained by the circulatory system.

Calabrese (1983) advanced a similar argument, noting that the regularity seen in metabolic rates and other physiological parameters is "so predictable, so consistent, it is inconceivable that it could have happened by chance. There clearly must have been some constant type of selection pressure to produce or shape these characteristics." He went on to suggest that temperature regulation is a logical possibility because "so many structural and physiological processes serve that end".

In spite of various complexities, it can be argued that heat loss across the surface has profound effects, not the least of which is placing a minimum size on mammals at that of a shrew, the smallest mammal. If there were a smaller mammal, the very high rate of heat loss would exceed the ability to gather adequate food to maintain a constant temperature (Gould, 1966). On the other hand, Calder (1984) suggests that the factor limiting the smallest mammalian and bird size to about 2 g is the heart rate required to deliver sufficient oxygen to shrews and humming-birds.

TABLE 1

Energy metabolism in dogs[a]

Weight (kg)	Metabolic rate (kcal/kg)	Metabolic rate (kcal/m^2)
31.20	35.68	1036
24.00	40.91	1112
19.80	45.87	1207
18.20	46.20	1097
9.61	65.16	1183
6.50	66.07	1153
3.19	88.07	1212

[a] Adapted from Schmidt-Nielsen (1970).

TABLE 2

Energy metabolism in various species[a]

Species	Weight (kg)	Metabolism (kcal/m^2)
Horse	441	948
Man	64	1042
Dog	15	1039
Hen	2	1008

[a] Adapted from Kleiber (1947).

According to Brody (1945), the surface law was not tested experimentally until 1883, after the development of the Rittenkofer calorimetric method and the Meeh surface-area measurement method. These developments allowed Rubner (1883) and Richet (1889) to investigate the law. Rubner used dogs and his results are shown in Table 1, which illustrates two important phenomena. One is that the metabolic rate per kilogram increases with decreasing size. The other is that when the metabolic rate is normalized to square metres of surface area, it is nearly a constant. Indeed, Rubner proposed that the heat produced by homeotherms in 24 h is nearly 1000 kcal/m^2. Rubner's work lent strong support to the surface area law for intraspecific comparison. In 1901, Voit published the interspecific data shown in Table 2, providing support for the use of the surface law in interspecific comparisons.

3.2.2 *The Measurement of Surface Area*

Obviously one of the key elements in the surface law is the measurement of surface areas in order to confirm eq. (5) and establish the value(s) of a_1. Numerous methods have been used including the following (Quiring, 1941).

- *Covering*—the surface of the animal is covered with cloth, paper or another substance. The area is then determined by weighing the material or using a planimeter.
- *Geometric*—the surfaces of the nearly cylindrical body parts are calculated from their lengths and circumferences. The head is treated as a cylinder.
- *Skinning*—the area of carefully removed skin is measured.
- *Photographic*—the area is calculated from the photographed silhouette.

- *Integration*—a roller of known surface area is used with a revolution counter.
- *Electrical*—the electrical capacitance of the body surface is determined.

Meeh (1879) reviewed the work of earlier investigators who made direct surface area measurements, and also obtained his own measurements of 16 human subjects aged 16–66 years. Meeh obtained values for the constant in eq. (5) which he wrote as

$$S = KM^{2/3}, \tag{7}$$

where M is in grams and S is in square centimetres. In terms of kilograms and square metres this becomes

$$S = 10^{-2}KM^{2/3}. \tag{8}$$

Meeh reported values of K of 12.3 for human adults and 11.9 for infants, and an agreement between calculated and measured surfaces areas of within 7%. Table 3 gives more recent values (some of which differ from Meeh's results) of K for several species.

Clearly there is a considerable range in the values of K both within and between species, because K can be constant only among animals of geometrically similar shape. To account for differences in shape, other workers have devised formulas which involve mass (M) and body length (L). These formulas are reviewed by Quiring (1941) and include the following:

The Stoelgner–Miwa formula: $$S = C(ML)^{1/2}, \tag{9}$$

where C is a constant.

The Lissauer formula: $$S = kML, \tag{10}$$

where k is a constant.

The Dubois–Dubois formula for humans: $$S = 71.74M^{0.425}L^{0.725}, \tag{11}$$

where surface area is in square centimetres, weight in kilograms and length in centimetres.

Using eq. (11), the range of surface areas for a 70 kg man is from 1.61 m^2 (height = 1.29 m) to 2.08 m^2 (height = 2.08 m); for a 10 kg child, the range is 0.35 m^2 (height = 0.29 m) to 0.76 m^2 (height = 1.29 m).

The Meeh formula with $K = 11$ (Quiring, 1944) predicts a surface area of 1.87 m^2 for a 70 kg adult and a surface area of 0.51 m^2 for a 10 kg child. Thus, the range given by the Dubois–Dubois formula for 70 kg adult of various heights gives only a ± 10% variation about the Meeh formula. For a 10 kg child, the range is somewhat larger (± 30–50%).

More recently, Takai and Shimaguchi (1986) used the covering technique to relate the body surface area to 15 anthropometric measurements (e.g. weight, height, chest circumference, head circumference, trunk length and ankle circumference). Measurements were obtained on 40 Japanese males, aged 18–26 years. Multivariate stepwise regression analysis revealed that 96% of the variance could be represented by using body weight, height and head circumference. The use of weight alone, as in eq. (5), accounted for 86% of the variance.

TABLE 3

Meeh's constant ($cm^2/g^{2/3}$) for several species

Animal	Value[a]	Range[b]
Mouse	8.95	6.3–11.4
Rat	9.0	7.15–11.6
Guinea-pig	—	7.1–10.4
Rabbit	—	5.7–10.0
Cat	—	8.7–10.7
Dog	6.67	9.9–12.3
Man	11.0	—
Monkey	11.7	—
Swine	9.0	8.8–15.3
Cow	9.0	9.3–11.0
Horse	10.0	9.0–10.5
Sheep	8.3	8.3–11.0
Whale	—	8.3–11.1

[a] From Quiring (1944).
[b] From Spector (1956).

Brody (1945) noted the difficulties involved in measuring surface area. He pointed out that there are several sources of error and that even on dead rats three investigators differed by as much as 60% (the difference between the minimum and maximum values) even though the same method (not specified) was used.

In view of the measurement uncertainties, one might argue that the choice of different Meeh constants for different species is not very rewarding and that one might as well choose a single value for K which gives an appropriate value for the surface area of the reference 70 kg person. Taking a value of 1.85 m^2 and substituting into eq. (8), we find that $K = 10.9$. This gives a value of 0.505 m^2 for a 10 kg child. Thus, we could either use eq. (8) for all species with $K = 10.9$ or, better yet in view of the uncertainties, $K = 11$. We could also use the formula given below (Chappell, 1985).

$$S = 1.85(M/70)^{2/3}\ (m^2), \tag{12}$$

TABLE 4

Representative surface areas (S) for several species (Chappel, 1985)[a]

Animal	Weight (kg)	Surface area (m^2)
Mouse	0.02	0.008
Chick	0.40	0.059
Rat (young)	0.10	0.023
Rat (mature)	0.40	0.059
Guinea-pig	0.75	0.090
Rabbit	2.00	0.170
Dog	10.00	0.510
Cat	2.00	0.170
Monkey	5.00	0.320
Human (child)	10.00	0.510
Human (mature)	70.00	1.850
Pig	60.00	1.670
Sheep	60.00	1.670
Cow	500.00	6.900
Horse	500.00	6.900
Whale	43 000.00	134.000

[a] Using $S = 1.85(M/70)^{2/3}$ m^2 where M is in kilograms.

where M is in kilograms. The other approach is to take a convenient choice of the Meeh constant, such as 10, to be used for all species (Calder, 1984). Clearly, that particular choice gives results differing by 10% from eq. (12).

Table 4 gives representative values for surface area for several species based on eq. (12). While Spector (1956) gives values of K for these and other species that have been obtained from direct measurements, in view of the wide ranges of these data and the uncertainties in the measurements, the choice of the simple formula given by eq. (12) will probably suffice for most applications where surface area is needed. Comparing the results in Table 4 and those of Pinkel (1958), who used Meeh's equation with appropriate values for the coefficient, gives differences no greater than ± 6%.

3.2.3 *Organ Sizes and Function, Body Fluids and Other Physiological Parameters*

In their original paper, Sarrus and Rameaux (1838) went on to establish interspecific relationships involving oxygen consumption, respiration rate, pulse rate, pulse volume and body size. These relationships were all based on the assumption that heat loss in a mammal is proportional to its body surface area. The argument about pulse rate involves the assumption that the heart volume or stroke volume of large and small animals should be proportional to body weight. The intensity of the blood current (volume × heart rate) is proportional to oxygen consumption, which is proportional to energy metabolism, giving a heartbeat frequency of mass$^{-0.33}$ if the surface area law holds. Calabrese (1983) illustrated these interconnections as follows:

> For example, heat production is dependent on the metabolism of glucose, which is contingent on the presence of oxygen, which is dependent on hemoglobin, which is dependent on red blood cell metabolism, which is dependent on blood flow, which requires a certain heart rate, which needs a certain lung volume and breaths per minute.

Brody (1945) pointed out that we owe a debt to Sarrus and Rameaux not only for originating the "surface law", but also for pointing to the way to develop interspecies comparisons of basic physiological factors linked to metabolism.

Some authors have reported that both size and function of a number of organs are proportional to body surface area. Quiring (1941, 1944) reported that the masses of the thyroid gland, adrenal bodies, heart, brain and liver are proportional to body surface area. Smith (1951) reported that kidney weight is proportional to body surface area. Smith (1951) also reported that a number of parameters related to renal function are nearly proportional to surface area. These include the number of glomeruli, urea ratio and creatinine clearance. Adolph (1949) reported that urea and creatinine clearance as well as nephron number are roughly proportional to surface area. Adolph, however, differed from Smith in finding that kidney weight is proportional to body weight to the power of 0.85.

Blood pressure (Hahn, 1952), blood volume (Griffin *et al.*, 1945), and cardiac output (Grollman, 1929) were initially reported to be proportional to surface area. For example, Baker *et al.* (1957) measured blood volume on 150 surgical patients using the ^{131}I method. They report that the blood volume had a mean of 2.68 l/m^2 with a range of about ± 20% of this figure (no standard deviation was reported). They found that the commonly accepted value of 80 ml/kg body weight was a good approximation only for those adults in the lower weight ranges (< 56 kg). These authors suggested that their data would not apply to young children. No pre-pubertal children were included in their study. Other authors (Smith, 1951) have reported that infants, even on a surface area basis, are quite different from adults. Glantz *et al.* (1976) found that young dogs (average weight 3.6 kg) had nearly twice the ouabain volumes of distribution per kilogram of body weight as adult

dogs (average weight 17 kg) and that this difference could be accounted for by the fact that young dogs have nearly twice the plasma volume and interstitial fluid space as adult dogs. This subject will be discussed in Section 4.2.2. More recent work has shown that blood volumes are proportional to body weight and not surface area (Prothero, 1980).

The changes in body water compartments during growth and the relationship with body surface areas were the subject of a study reported by Friis-Hansen (1961). He reported the results of a study that he and his co-workers performed which involved subjects ranging in age from new-born to 15 years. Values were obtained for total body water (TBW), extracellular water (ECW), and intracellular water (ICW). Some body specific gravity measurements were also made; these are of some interest because the derivation of eq. (5) is based on the assumption of constant specific gravity. Variation in the specific gravity was about 5% in the five infants measured, the experimental error was not reported. Friis-Hansen and his co-workers found that ECW was proportional to surface area, i.e.

$$\mathrm{ECW} = 0.583W^{0.678}, \tag{13}$$

(where W is mass in kg) but this was not the case for TBW and ICW which were given by

$$\mathrm{TBW} = 0.843W^{0.891}, \tag{14}$$

and

$$\mathrm{ICW} = 0.309W^{1.094}. \tag{15}$$

Friis-Hansen suggested that the relationships found between kidney function and basal metabolism might be simply coincidence. He pointed out that if kidney function depends on ECW, and ECW is a function of surface area, then the relationship between kidney function and surface area might be viewed as a secondary or indirect one.

3.3 APPLICATIONS TO DRUG METABOLISM AND TOXICITY

The problem of calculating the appropriate dose of a drug for humans of different age, sex, weight and shape has concerned physicians and pharmacologists for at least a century and a half, and probably longer. Butler and Richie (1960) reported that in 1830 Hufeland proposed "on the basis of clinical experience a scale of doses of drugs according to size, expressed as a percentage of adult dose being closely proportional to body surface areas". Dreyer and Walker (1914) reproduced some of Hufeland's results (which they claim date from 1818). Hufeland based the dosage for a child relative to the adult dose on age; for example, he proposed that a one-year-old child should receive a dose one-quarter that of a 21-year-old. Using average weights of 61 kg for the 21-year-old and 9 kg for the one-year-old, the use of body weight equivalence would predict one-seventh, whereas surface area equivalence would predict one-quarter for the one-year-old relative to the 21-year-old.

It is difficult to determine who next proposed the use of surface area to calculate drug dosage. One of the earliest papers was by Moore (1909), who noted that clinical evidence did not support body weight equivalence. He argued that body surface area as given by weight to the power 2/3 was the proper approach. His explanation was that many drugs (particularly heavy metals such as arsenic) attack cells spread out on a surface. It was his contention, however, that this meant that the blood concentrations reached in larger animals would be smaller (presumably because he assumed that blood volume was proportional to weight and that the concentration was the dose divided by the blood volume). Therefore, he argued that the surface law "sets a limit to our power of applying therapeutic agents against disease in larger animals". This, he argued, explained why it was possible to cure certain protozoan diseases in small animals using, for example, organic arsenicals or arsenic and mercury, but not possible to cure the diseases in larger animals.

Dreyer and Walker (1914) proposed the use of surface area. But, in contrast to the previously discussed work (Moore, 1909), they argued that this method of calculating drug dosage would lead to equal blood concentration because evidence at that time suggested that blood volumes of homeotherms are proportional to their surface areas. Both they and Moore were calculating blood concentrations incorrectly (as we will show in Section 5.2.2) by assuming that the concentration in the blood at any time after administration was equal to the dose divided by the blood volume. Moreover, they performed a number of surface-area measurements (they do not discuss their methods) and claim to have shown that surface area is more nearly proportional to the mass to the power 0.72 and this, they claim, was also the case with blood volume.

An interesting example of the problems of extrapolation was reported by West and Pierce (1962). The investigators wished to study the effect of lysergic acid diethylamide (LSD) in an elephant. They chose the dose of LSD that produced rage in a cat (0.1 mg/kg) and extrapolated that dose to the elephant by multiplying by a body weight of 2970 kg, to arrive at 297 mg of LSD as the dose for the elephant. The elephant went into a frenzy of running and trumpeting, then quickly fell into convulsions and died 1 h 40 min after the injection. The authors concluded that "It appears that the elephant is highly sensitive to the effects of LSD..." Harwood (1963)

suspected that the elephant died from an overdose of LSD and noted that had the surface area law been applied to the dose required to produce psychosis in humans (0.1–0.2 mg), the result would have been about 3 mg instead of 297 mg. He further suggested that a safety factor of 10 should have been used to determine the initial dose. There is no way, of course, of knowing whether the use of a 0.3 mg dose would have prevented the unfortunate result.

Crawford *et al.* (1950) reported on a study of approximately 60 patients at the Massachusetts General Hospital. They divided the patients into four groups based on surface area (as calculated from a nomogram using height and weight). These groups were infants (0.15–0.49 m^2), young children (0.5–0.99 m^2), older children and adolescents (1.0–1.49 m^2), and adults (1.5–2.0 m^2). They excluded patients with severe gastrointestinal, cardiovascular or renal disturbances. These patients were, as a part of their treatment, receiving either sulphadiazine or acetylsalicylic acid at regular intervals of 8 h or less and had been on that schedule for at least 48 h. Blood samples were drawn and analysed for the concentration of the drug that each patient had been receiving. The doses were calculated and plotted against the blood concentrations as illustrated in Figures 1 and 2.

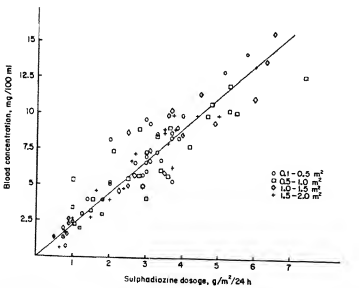

FIG. 1. Relation between blood concentration and sulphadiazine dose in patients of varying size (from Crawford *et al.*, 1950; reproduced with permission of the publisher).

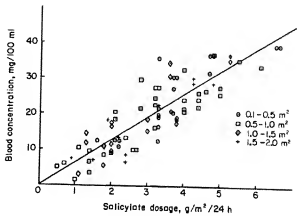

FIG. 2. Relation between blood concentration and salicylate dose in patients of varying size (from Crawford *et al.*, 1950; reproduced with permission of the publisher).

Crawford *et al.* (1950) found a linear relationship between blood concentrations and doses based on surface area. The calculations revealed that a dose level of 4.25 g/m^2/day of sulphadiazine results in a blood level of 10 mg/100 ml; the standard deviation did not exceed 15% at any dose level. In the case of acetylsalicylic acid a linear relationship was also found. They observed that a dose of 3.2 g/m^2/day resulted in a mean of 20 ± 5 mg/100 ml. They also observed that accepted dosages (e.g. the average analgesic dose for morphine at that time) of several commonly used drugs conformed to the surface area law. This was also the case for the volume of single blood transfusions. Table 5 illustrates their results for some of these drugs. It is clear that the doses per surface area are more similar than the doses per kilogram of body mass.

Crawford *et al.* (1950) reported that the use of surface area to calculate drug dose had been given a clinical trial of more than a year on the Children's Medical Service of the Massachusetts General Hospital, where it was found applicable throughout the size-range of patients. An extensive list of therapeutic agents (names not given) was tried, and no exceptions had been found to that time.

Talbot *et al.* (1953), Snively (1957), Baker *et al.* (1957) and Talbot and Richie (1959) also reported on the use of surface area to determine the dosages of therapeutic fluids, blood and electrolytes. These investigators all found that surface area was preferable to body weight in dosage calculations.

TABLE 5

Comparison of standard dosages on a surface area basis (from Crawford *et al.*, 1950)

Weight (kg)	Morphine dose			Thyroid dose			Blood dose		
	mg	mg/kg	mg/m^2	mg	mg/kg	mg/m^2	ml	ml/kg	ml/m^2
5	2.0	0.4	7.4	20	4.0	74	100	20	370
10	3.0	0.3	6.7	30	3.0	67	150	15	330
30	7.0	0.23	6.4	60	2.0	55	300	10	270
50	10.0	0.2	6.7	90	1.8	60	500	10	330
70	12.5	0.18	7.2	90	1.3	58	500	7.1	290
Average dose/m^2			6.9			63			320

Nevertheless, it is important to note that the use of surface area to calculate dosage has had dissenters. In Section 4 we will discuss at length the controversy regarding the surface rule, but it is of interest to note here that the work of Talbot was criticized by Forbes (1959), who suggested that the surface area approach should simply be considered as an empirical method so that there would be "no tendency to treat the use of surface area as a basis for calculating dosage as though it were a universal scientific principle". Oliver *et al.* (1958) claimed that the use of surface area to compute parenteral fluid dosage was "based on confused logic and inadequate observation." They claimed that a simple rule-of-thumb based on body weight (but using different values based on age) gave the same results. They also pointed out that several authors had reported that a review of data on energy metabolism showed that energy metabolism per square metre was not a constant (this will be discussed in Section 4).

The application of the surface area rule to drug dosage calculation clearly involves toxicity as an important consideration. Cancer chemotherapy focussed attention on the extrapolation of toxic effects because these drugs are often effective only at levels near or above toxic levels. As a result, the extrapolation from animal studies to human treatment is critical. The importance of the cancer chemotherapy data to this study is that more information exists about the toxicity of these drugs to humans under controlled conditions than about any other chemicals.

Pinkel (1958) was apparently the first to apply the surface law to anti-cancer drug dosage calculations. He reviewed the literature on several drugs to determine the "generally accepted" levels for therapeutic doses in animals and humans. The drugs he considered were mechlorethamine, methotrexate, 6-mercaptopurine, actinomycin D, and triethylenethiophosphoramide (TSPA). Surface area for animals was calculated from Meeh's formula (eq. 7) and the Dubois–Dubois formula (eq. 11) was used for humans. Pinkel found that the generally accepted doses in terms of milligrams per square metre per day were remarkably similar between and within species. The differences were generally less than 20%. The only exception was 6-mercaptopurine, where the same dose per unit body weight per day (3 mg/kg/day) was used for humans ranging in weight from 8 to 70 kg, leading to an adult dose that was 70% larger than the dose for a child. He suggested that this might explain the observation that, when given the usual dose of this drug, adults generally develop earlier and more severe toxicity than do children. Pinkel also suggested that the mechanism for the relationship to surface area could be the relationship of renal function to surface area, because three of the five chemicals were excreted by the kidneys.

Shnider (1961) discussed some of the problems encountered in early human trials with anticancer agents. One problem involved the drug 6-azauracil. Pharmacological studies showed that animals (mice, rats, cats, dogs, and monkeys) could tolerate doses as high as 90 mg/kg/day with no definite toxic manifestation; however, humans developed significant toxicity at 4.5 mg/kg/day. Unfortunately, there are no data in this paper to indicate which animals tolerated the 90 mg/kg/day dose but, using surface area for conversion, that dose in a mouse would be equivalent to a dose of 6 mg/kg/day in a human.

Clearly there is a considerable importance in obtaining accurate, quantitative methods for extrapolating the results for anticancer drugs from animals to humans. Since these agents are not generally effective at doses much below toxic levels, the use of large safety factors is counter-productive. On the other hand, there is little margin of safety, so over-estimates are equally undesirable. In a discussion of the problems of extrapolation, Rall (1969) noted that "The problem of predictability lies primarily in the fact that the stakes are so high and not that the systems are so bad."

The classic study in this regard was made by Freireich *et al.* (1966). They reviewed published and unpublished data on the toxicity of various anticancer drugs to animals and humans, and compared the maximum tolerated dose (MTD) for humans with the LD_{10} (dose required to kill 10%) or MTD for animals. It was necessary to use data from different routes. They did attempt to use a similar dosage schedule by a normalization procedure whereby, for example, the doses from a study using a 10-day dosage period were doubled to simulate a 5-day dosage period. They then calculated the surface area by using Meeh's law (eq. 7) with K values obtained from the literature. A conversion factor was developed so that they could convert

directly from dose per unit body weight to dose per unit surface area. This factor (km) was given by

$$(\text{dose in mg/m}^2) = km\ (\text{dose in mg/kg}). \tag{16}$$

From eq. (8) we see that

$$km = \frac{10^2 M^{1/3}}{K}, \tag{17}$$

where M is the mass in kilograms.

If we assume that SAE exists then we translate from animal to human dose by

$$\text{dose in man (mg/kg)} = \text{dose in animal (mg/kg)} \times \left[\frac{km\,(\text{animal})}{km\,(\text{man})}\right] \tag{18}$$

Table 6 gives the values of K and km used by Freireich *et al.* (1966). For a mouse we find that

$$\text{dose in human adult (mg/kg)} = \left[\frac{\text{dose in mouse (mg/kg)}}{12.3}\right] \tag{19}$$

Thus, if the LD_{10} for mice is 100 mg/kg then the MTD for a 60 kg human is assumed to be 8.4 mg/kg on the basis of eq. (19). This equation was then tested against the data obtained from the literature review. Figure 3 shows the results of 18 drugs. Of course, BME implies a one-to-one relationship as shown in Table 6. Clearly, the data in Figure 3 follow SAE much more than

TABLE 6

Conversion factors from mg/kg to mg/m^2 (Freireich *et al.* 1966)

Species	Weight (kg)	K	km	Surface area equivalence[a]	Body mass equivalence
Human	60.00	10.6	37.0	1 to 1	1 to 1
Mouse	0.02	9.0	3.0	12 to 1	1 to 1
Rat	0.10	9.0	5.2	7 to 1	1 to 1
Hamster	0.05	9.1	4.1	9 to 1	1 to 1
Monkey	2.50	11.8	11.5	3 to 1	1 to 1
Dog	7–8	10.1	19.0–19.8	2 to 1	1 to 1

[a] Ratio of km (man) to km (animal), rounded to the nearest integer.

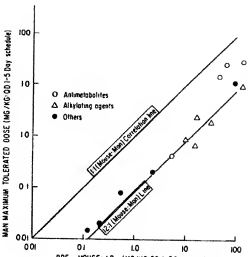

FIG. 3. Comparison of toxicity data on anticancer agents for the mouse and man. The 1 : 1 line is the result for BME and the 12 : 1 line SAE (from Freireich *et al.*, 1966; reproduced with permission of the publisher).

BME; the factor of 12.3 is the reason for the offset of the two lines. Similar results were found for the other species.

Freireich *et al.* (1966) also looked at other models. One model differed from that above in that instead of assuming

$$\text{dose in man (mg/m}^2) = \text{dose in animal (mg/m}^2) \tag{20}$$

it assumed that

$$\text{dose in man (mg/m}^2) = A_i \times \text{dose in animal (mg/m}^2), \tag{21}$$

where $i = 1 \ldots 6$ are the animal species and A_i is an empirically determined constant. Equation (20) was called Model 1 and eq. (21) Model 2. Model 3 involved a multispecies equation using multiple regression:

$$\text{dose in man (mg/m}^2) = \Sigma\, A_i \times \text{dose in animal (mg/m}^2). \tag{22}$$

The predictive capability was somewhat better for Models 2 and 3 than for Model 1. But Freireich *et al.* (1966) did not feel that Model 2 offered significant advantages over Model 1. Model 3, of course, has a disadvantage in that it requires data from all the animal systems to make a prediction.

Rall (1969) noted that for methotrexate, the integrated plasma concentration time function (the area under the curve or AUC) after the same dose in mouse and humans is 10 times higher in humans than in the mouse. [The importance of the AUC in cancer chemotherapy has been noted more recently by Collins *et al.* (1986)]. This is close to the factor of 12.3 seen in eq. (19). The role of the area under the concentration curve is discussed in Section 5.2.2. Rall also noted that some toxic responses in humans (such as bone marrow and kidney abnormalities) are easier to predict from animal data than others (e.g. CNS and dermatological effects).

Several authors have reviewed the problems associated with the use of surface area for calculating the dose of anticancer drugs. These include Schein *et al.* (1970), Homan (1972), Mantel and Schneiderman (1975), Hoel (1975) and Goldsmith *et al.* (1975), and, more recently, Collins *et al.* (1986). They have discussed numerous concerns and pointed out several complications that must be considered. These include relatively better predictive capability for some toxic effects than others, and the observation (Schein *et al.*, 1970) that while some of the same effects may occur in humans and animals, the order of appearance (as a function of dose) may not be the same.

There is a considerable data-base on human toxicity of chemotherapeutic drugs which facilitates the comparison of BME and SAE in the extrapolation of toxicity data. It is certainly true, then, that such comparison will be less easy with other chemicals; however, it is possible to obtain some indications of the comparison. Chappell (1984, 1985) has compared these approaches for several trace elements. The two best examples are for molybdenum (Mo) and barium (Ba).

Molybdenum is generally accepted as a micronutrient with normal daily intakes for humans of 0.12–0.24 mg/day, depending on age, sex and income (Tsongas *et al.*, 1980). The toxic effects of Mo in animals include decreased growth rates, diarrhoea, alopecia, and anorexia at lower levels, while higher doses damage the kidney, liver, reproductive systems and bones. Because the toxic dose is less in livestock (cattle and sheep) than in small animals, the former have always been considered especially sensitive to Mo toxicity.

The use of BME to extrapolate Mo data leads to many inconsistent results; for example, subacute effects are seen in rats at doses (in relatively soluble compounds) of 1–2 mg/kg body weight (Gray and Daniel, 1964). Such doses are well above the levels (0.14 mg/kg per day) reported by Koval'skiy *et al.* (1961) to have adverse effects on humans. Underwood (1976) estimated that the minimum daily requirement for rats is less than 0.2 ppm in their diet (again, in relatively soluble compounds), corresponding to 0.02 mg/kg body weight per day. BME leads to an estimate of 1.4 mg/day for a 70 kg adult human, which is nearly 10 times normal daily

TABLE 7

Comparison of predicted and actual thresholds for subchronic toxicity of molybdenum

Species	Weight (kg)	Surface area (m^2)	Predicted LOAEL[a] (mg/kg)	Actual LOAEL (mg/kg)	Actual LOAEL (mg/m^2)
Rat	0.1	0.023	—	1–2	4–9
Guinea-pig	0.3	0.05	1.5	1–10	6–60
Rabbit	2	0.17	0.7	1–10	11–120
Pig	60	1.7	0.2	2	71
Man	70	1.85	0.2	0.14–0.20	5–8
Cow	500	6.9	0.1	0.07–4.00	5–290

[a] LOAEL = Lowest observed adverse effect level.

intakes. Since Mo deficiencies are not seen in humans, this is clearly an erroneous estimate. When SAE equivalence is used, the subacute dose in rats extrapolates to 0.1 mg/kg per day and the minimum daily requirement estimate to *less* than 0.16 mg/day, which is more in line with normal daily intakes.

The lack of uniformity in the toxicity literature makes comparisons difficult. Tables 7 and 8 give Chappell's (1985) interpretation of the literature (for relatively soluble compounds such as sodium molybdate) in terms of the LOAEL for subchronic toxicity and the lethal doses (LD_{50}).

TABLE 8

Comparison of predicted and actual lethal doses of molydbenum for various species (Chappell, 1985)

Species	Weight (kg)	Surface area (m^2)	Lethal dose (mg/kg/day)	
			Predicted	Actual
Rat	0.1	0.023	—	125
Guinea-pig	0.3	0.05	90	70
Rabbit	2	0.17	45	50–60
Man	70	1.85	14	?
Cow	500	7	7.6	4–10

The rat data is used to predict (with SAE) LOAELs and LD_{50}s for other species and compare predictions with the observed results, where they exist. No acute poisoning of humans has been reported. With the exception of the pig, the use of SAE leads to good agreement between prediction and observation.

Barium is absorbed through the nasal mucosal membranes, lungs and gastrointestinal tract as a result of the solubility of Ba compounds: $BaCl_2$ is quite soluble in water and readily absorbed; barium carbonate is poorly absorbed and has a low solubility. Table 9 summarizes the lethal dose data for $BaCl_2$ and compares the lethal dose in milligrams per kilogram with that in milligrams per square metre. It can be seen that the use of SAE to extrapolate the lethal dose from any of the animals listed would lead to a result that is within 50% of the observed lethal dose for humans. The use of BME and the rat data would overestimate the human dose by almost a factor of six.

Chappell (1985) noted that when the same comparison is made for barium carbonate, which in milligrams per kilogram is nearly an order of magnitude less toxic to rats and humans, the comparison is not as good as seen in Table 10. Since barium carbonate is poorly soluble, this discrepancy may be the result of not knowing the amount absorbed into the body in relation to the amount ingested.

Chappell also compared SAE and BME for vanadium, arsenic, chromium, nickel, and selenium. SAE gave generally better predictability than BME for several vanadium compounds, arsenic trioxide, hexavalent chromium, selenate and selenate compounds. It did not give very good

TABLE 9

Comparison of lethal dosages[a] of $BaCl_2$ for various species[b] (Chappell, 1985)

Species	mg/kg	mg/m²
Rat	44	190
Rabbit	38	450
Cat	33	390
Dog	10	200
Guinea-pig	36	300
Man[c]	8	300

[a] LD_{100}.
[b] Adapted from Venugopal and Luckey (1978).
[c] Solliman (1957).

TABLE 10

Comparison of lethal doses of barium carbonate[a] (Chappell, 1985)

Species	Toxicity	Lethal dose	
		mg/kg	mg/m²
Mouse	LD_{100}	112	280
Rat	LD_{100}	449	2000
Guinea-pig	LD_{100}	85	710
Rabbit	LD_{100}	340	4000
Man	MLD[b]	56	2000

[a] Adapted from Venugopal and Luckey (1978).
[b] Minimum lethal dose.

agreement for sodium arsenate, trivalent chromium compounds, and nickel carbonyl.

Mantel and Schneiderman (1975) pointed out that if the dose is expressed in terms of concentrations in food, drink or air, the surface area rule for chronic exposure is equivalent to assuming equal concentrations in these media, because the requirements for all three are roughly proportional to surface area.

3.4 DISCUSSION

The surface area law has an interesting and colourful history. The fact that it seemed to explain why the metabolic rate per kilogram of small animals was much larger than that of larger animals made it very attractive as a model. In fact, this explanation became so popular and widely accepted that, as pointed out by Kleiber (1947), data which disagreed with the surface law were assumed to be incorrect or, in some cases, somehow forced into agreement.

Much of the early work concerning the surface law involved a greater effort to develop measurement apparati, derive formulae, or develop interpretations than to actually test the validity of the idea (Kleiber, 1947). It is of interest that Dreyer and Walker (1914) proposed that a somewhat different power, 0.72, of the weight was involved. But generally, even as recently as the mid-1970s, investigators assumed the surface law in making comparisons with body weight equivalence for drug dosage calculations.

Certainly the experience of nearly a century and a half has shown that body weight equivalence does not generally hold (Moore, 1909; Dreyer and Walker, 1914; Pinkel, 1958; Freireich *et al.*, 1966; etc.). There is considerable evidence that, for most chemicals, the toxicity to humans in milligrams per kilogram is approximately 10 times greater than that for mice or rats. As a result, the assumption of body weight equivalence would generally use up all of the safety factor of 10 that is often used to reflect the uncertainty in animal-to-human extrapolation. On the other hand, the existence of several formulae for surface area and the difficulty of making actual measurements of surface area have clouded the interpretation of the surface law. There has been a considerable controversy about the mechanisms by which the total body surface area could influence drug metabolism. Done (1964) suggested that the linkage of the surface area with dosage was a semantic *faux pas* which has led to unnecessary problems of interpretation. Instead, he proposed an empirical approach whereby surface area is considered as a surrogate representing a more appropriate power function of the body weight than that given by BME. In the next section, we will show that this empirical approach had already been applied for over 30 years by the time Done (1964) made this statement.

The surface law did, however, play an important role in the development of comparative physiology by focussing attention on a unifying theme. One result of this effort was that instead of interpreting the differences of equivalent doses in milligrams per kilogram of body weight as a reflection of different responses in each species, the use of the surface law gave an approach for viewing this phenomenon as a reflection of interspecies similarities.

Rall (1979) has noted that the scientific community can be conveniently divided into "lumpers" and "splitters". The lumpers tend to find patterns while the splitters tend to find differences. Both sides, he noted, are important to the advance of science. The surface area law gave considerable support to the lumpers' cause.

Is the "surface law" a general truth or a fortunate coincidence for some situations? We may never know the answer to that question. But the work done by Freireich *et al.* (1966) and others has given strong support to the use of SAE in preference to BME. Since SAE will give a more conservative estimate from a toxicological point of view when extrapolating data, it is probably the preferable choice if no data are present to support an alternative choice.

In view of the uncertainties involved, there seems to be no reason to develop elaborate formulae for surface area with numerous empirical coefficients as was done by Freireich *et al.* (1966) and others. If we view the surface area as a surrogate for complex and incompletely understood mechanisms, we can simply choose a convenient formula such as in eq. 12; that is, since the constants in Meeh's equation (see Table 3) are not very different between species, it is the value of the exponent (0.67) in the surface law which is the overriding factor. In the next section, we will show that for several decades the argument in comparative physiology has centred around the value of the exponent.

Since surface area equivalence gives a more conservative estimate, it provides a more prudent approach for calculating dosage and is a valid argument against body weight equivalence. Moreover, there does seem to be more evidence supporting the use of surface area, or at least an exponent of 0.7 (which, in view of all the uncertainties involved, is a more reasonable number of significant figures than 0.67), than the use of body mass (i.e. an exponent of 1.0).

4 Allometric Relationships and Biological Similarity

4.1 INTRODUCTION

In this section we discuss an approach that was given the name "allometry" by Julian Huxley and G. Tessier in 1936 (Huxley and Tessier, 1936). Allometry (literally "of a different measure"; McMahon and Bonner, 1983) simply proposes the equation

$$y = bx^k, \tag{23}$$

connecting a dependent variable, y, with the independent variable, x, and includes the surface law concept as a special case. In the context of these considerations, x will generally be the body mass, M, and y is the parameter of interest, e.g. liver weight, energy metabolism or LD_{10}. The surface area case is represented by $k = 2/3$, if we assume that surface area is proportional to the two-thirds power of mass.

Davidson *et al.* (1986) in their review of the literature attempted to codify their conclusions in four "principles" with accompanying "corollaries". The first of these was as follows:

PRINCIPLE. The biological basis for extrapolation of experimental data from one animal species to another is founded in the overwhelming anatomical, physiological and biochemical similarities among species, with the recognition that singular differences may occur within one species and not in others. Morphological and biological functions are quantitatively related among species by body weight (W) according to the general allometric equation

$$Y = aW^n$$

where the values of a and the exponent n are distinctive of the biologic function Y. Hence, the morphologic and biologic functions themselves are related by the general equation

$$Y = a(X)^n$$

where Y and X are two functions.

COROLLARY. $W^{1.0}$ and $W^{0.67}$ as bases for extrapolation of experimental data have no unique justification as intrinsic common denominators of biologic similarity among species. Additionally, the traditional terms "mg/kg body weight basis" and "on basis of body surface area" are commonly misunderstood as connoting two qualitatively differing bases of extrapolation, whereas both are encompassed by the general allometric equation, $Y = aW^n$, and differ quantitatively only as $W^{1.0}$ and $W^{0.67}$, respectively.

While we agree, we will see that there are many authors who would not agree with the above statements.

The allometric approach has been used in a very large number of fields including paleontology, comparative physiology and pharmacokinetics. Gould (1966) has proposed five major classes of allometry: ontogenetic allometry, evolutionary allometry, intraspecific allometry involving members of a single population, intraspecific allometry involving races or subspecies, and interspecific allometry. Gould (1966) defines allometry as the study of size and its consequences. In its broadest sense, it designates differences in proportions (morphological, physiological or chemical) that are correlated with changes in the size of the organism or its parts, whether these differences arise from ontogeny or phylogeny. The study of the extrapolation of toxicity data from animals to humans falls under this broad definition.

Galileo has sometimes been described as the first allometrist because of his observations on the breaking strength of structures and the relationship between the size of objects and their supporting structures. In his *Dialogue of the Second Day* (1638), Galileo demonstrated that the diameters of supporting limbs of animals and trunks of trees must become proportionately larger as their mass increases, in order to support their weight (in fact, the square of the diameters must increase as the weight to the first power). Thus, the mass of the supporting limbs increases at a rate faster than that of the total body mass. In spite of these early contributions allometry, basically the application of curve-fitting to physiological data, did not really develop until the method of linear regression on logarithmically transformed data became widely used and accepted.

In this section, we will review the literature by discussing how the use of regression analysis led to somewhat different conclusions about energy metabolism than those discussed in the previous section. From one point of view, these differences were not very large, involving exponents of about 0.75 instead of 0.67. From a theoretical point of view, the differences were considered very large indeed and have inspired many investigators to carry out further empirical work to establish these exponents better. Even today, there is an argument about whether the exponent is 0.67 or 0.75. In addition to extensive empirical work, numerous investigators have attempted to develop theoretical justifications for the allometric equations. Many of these are analogous to the approach that engineers take in scaling from prototype-size systems to full-scale systems using the theory of similarity (dimensional analysis). These engineering approaches usually involve developing an appropriate constant which is invariant (unchanged) under size change and is a function of the basic system parameters.

Finally, we illustrate a few applications to toxicity data. One application involves the same data base considered by Freireich *et al.* (1966) whose work was discussed in Section 3.3. Mordenti (1986a) applied regression analysis to this data and found exponents that vary from 0.60 to 0.87.

4.2 LITERATURE REVIEW

4.2.1 Differences from Surface Area Law

In Section 3.2, we discussed many of the problems inherent in measuring surface area; we also mentioned the wide discrepancies in surface area measurements reported by Brody (1945). As early as 1914, Dreyer and Walker had reported that their surface area measurements indicated that surface area is proportional to mass to the power 0.72 rather than the two-thirds power. It is not surprising that the widespread application of the surface area law in the form of $M^{2/3}$ was found to be less generally valid than first thought; it is interesting, however, that these differences from the surface area law are not more widely known. Even as the surface area law has been rediscovered from time to time, the same is true of the information that there are discrepancies from this law — indeed, many discrepancies.

Probably the most important events regarding the deviations from the surface law were the nearly simultaneous publications of studies by Kleiber (1932) and Brody and Proctor (1932), in which it was reported that the energy metabolism of homeotherms is not proportional to $M^{2/3}$ but to $M^{3/4}$ (Kleiber, 1932) or $M^{0.734}$ (Brody and Proctor, 1932). Figure 4 illustrates the extraordinary range of the fit obtained by Brody (1945). Kleiber showed in particular that the metabolic rate per unit surface area has a highly significant positive correlation with body weight or mass, whereas when metabolic rate is divided by $M^{3/4}$ the correlation disappears. His calculations

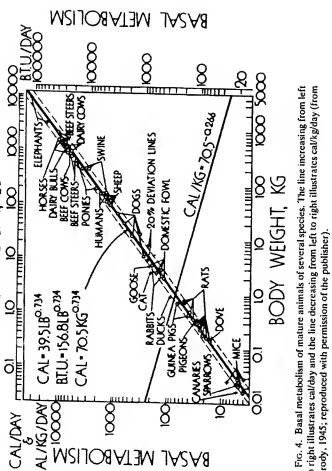

FIG. 4. Basal metabolism of mature animals of several species. The line increasing from left to right illustrates cal/day and the line decreasing from left to right illustrates cal/kg/day (from Brody, 1945; reproduced with permission of the publisher).

revealed that fasting homeotherms produce 3 kcal/kg$^{3/4}$/h. These calculations used data from species ranging in weight from a 10 g mouse to a 16 ton elephant, thus giving rise to the "mouse to elephant" curve. These results were accepted by some parts of the scientific community at that time. Indeed, the National Research Council (NRC) Committee on Animal Nutrition (1935) officially endorsed $M^{0.73}$ as the most appropriate relationship of mass to energy metabolism.

It appears that the surface law had such a strong theoretical appeal that it was virtually unquestioned from 1838, when it was first proposed, to about 1930. While a great deal of effort was spent on explaining why some data didn't fit the surface area law, very little time was spent trying to verify the law itself. In essence, Kleiber, Brody and Proctor were the first to attempt to find the curve best fitted to the data.

Once the apparent discrepancies were noted, several investigators reported relationships that did not involve $M^{2/3}$ or, for that matter, $M^{3/4}$. In a landmark paper by Adolph (1949), data for several physiological parameters were reviewed. The exponents in the allometric equations ranged from 0.08 (diameter of the renal corpuscle) to 1.31 (myoglobin weight). Table 11 lists the exponents found for certain parameters.

While Brody, Kleiber and others reported a good fit of energy metabolism of mature animals to the three-quarters power of the mass, this relationship does not appear to be valid when animals at different stages of growth are used. Brody (1945) pointed out that such a constancy should not be anticipated because there is necessarily an energy cost of growth, apart from the basal metabolism, and that this would be expected to change with time.

TABLE 11

Exponents relating physiological parameters to body mass for mammals[a]

Parameter	Exponent (k)
Water intake (ml/h)	0.88
Urine output (ml/h)	0.82
Inulin clearance (ml/h)	0.77
Basal O_2 consumption (ml STP/h)	0.73
Breath duration (h)	0.28
Haemoglobin weight (g)	0.99
Kidneys' weight (g)	0.85
Blood weight (g)	0.99

[a] From Adolph (1949).

He also noted that early prenatal life is not homeothermic and that the neuro-endocrine system does not attain maturity until late in the developmental cycle.

In terms of surface area considerations, the organism changes from a relatively simple spherical shape to very complex geometries involving membranes, renal tubules, etc. Indeed, Gould (1966) notes that the problem of the decreasing surface to mass ratio as a function of increasing size can be "escaped" by three mechanisms:

- The differential increase in surface by geometrical complexity such as branching and convolution.
- The differential increase of surfaces by a gross change in shape such as flattening.
- The incorporation of less metabolically active material such as bone.

As a result, it seems reasonable to assume that metabolism per unit surface area or the two-thirds power of mass would start off at a relatively low rate, rise with growth, level off at maturity and eventually decline with old age. Brody (1945) illustrated this process for rats (see Fig. 5).

When the data in Figure 5 are put on a log–log plot, with energy metabolism plotted versus body mass, a sequence of straight lines with different slopes are obtained. Some of the breaks between lines of different

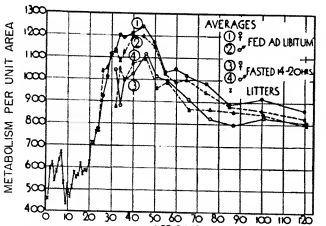

FIG. 5 Metabolism per unit area in rats as a function of age (from Brody, 1945; reproduced with permission of the publisher).

TABLE 12

Allometric equations for human basal metabolism at different ages (Brody, 1945)

Age range (years)	Male exponent	Female exponent
0–3	1.02	1.05
3–16	0.59	0.64
16–31	0.65	0.46
31–60	0.56	0.38
Over 60	0.55	0.57

slope can be related to developmental milestones such as weaning. Similar results are seen for humans — curves that have exponents varying with age. Table 12 illustrates the results from Brody (1945). Thus, while the metabolic rate initially rises quickly as a function of increasing mass, it tends to drop with time and eventually, according to Brody (1945), the metabolism of mature humans approaches the general curve relating metabolism to weight in mature animals of other species. This idea of describing physiological parameters as a function of mass in growing animals has been termed ontogenetic allometry by Gould (1966) or growth curves by Bertalanffy (1957) and others. The study of this phenomenon was inspired by the work of D'Arcy Thompson (1917) and Sir Julian Huxley (1932).

Brody also reported results on prenatal heat production in pregnant animals to obtain the heat increment of gestation. He noted that there are several types of energy expenditure associated with gestation, including the maintenance of the pregnant uterus, the work of growth, the increased work by the maternal circulatory, respiratory and excretory mechanisms, and the endocrine influences of the maternal metabolism. He reported that the heat increment was proportional to birth weight to the power of 1.2. The considerations given above of changes in metabolism with age and with pregnancy have potential consequences for the extrapolation of toxicity data from animals to young children and pregnant women.

In view of the divergence from the surface area law, some investigators (Brody, 1937; Kleiber, 1947) proposed that the exponent in the allometric equation for energy metabolism defines a physiological or metabolic body mass or weight, the idea being that not all the mass of the animal is equally involved in metabolism. This concept also incorporates the point made by Galileo when he noted that the strength of the limbs of an animal would be

proportional to the cross-sectional area, which either must grow as fast as the animal's weight or must employ a stronger material; unless the proportions are changed, relatively more structural (and therefore less metabolically active) material must be added as the animal grows.

Thus, at least in one area which might be called comparative physiology, the attention shifted from the surface area relationships to allometric equations which were obtained empirically using regression analysis.

4.2.2 *Empirical Basis*

One can, of course, simply treat the surface area law as a special case of allometry where the exponent is taken as 2/3. The view of most investigators, however, has tended to be that since the exponents observed were generally different from 2/3, the surface area explanation had to be replaced by an alternate theoretical basis. This feeling increased as more evidence was obtained for allometric equations having a different form than expected by the surface area law. Such evidence was obtained not only for basal metabolism, which was the original physiological parameter that provided interest in this general topic, but also for parameters relating to circulation, renal function, respiratory function and organs' weights.

The fact that other parameters were tied with metabolic rate was not surprising. As pointed out earlier, respiration, blood circulation and metabolic rates are physiologically related. Schmidt-Nielsen (1984) gave an elegant discussion of the interrelationship of these variables via allometry. The fact that there are several parameters that are independent of body mass, such as haemoglobin concentration, blood viscosity, red blood cell size, blood pressure and capillary diameter, is related to the fact that blood volume is proportional to body mass, to the delivery of oxygen to tissues and to the supply of substrate to be oxidized. The consistency of these relationships among different species suggests the evolution of a system adjusted to the maximum transport capacity of oxygen while minimizing the work required for pumping.

Much of the work done on interspecies scaling involved the systematic collection and evaluation of data from the literature for use in the linear regression models; for example, Brody (1945) used data in species ranging from the dwarf mouse to the elephant to obtain a basal metabolism curve with an exponent of 0.73. While some large deviations were found for individual species such as the dwarf mouse and the elephant, this was explained away in terms of either a lack of normal metabolism (for the dwarf mouse) or technical difficulties in measuring the parameters (e.g. the elephant metabolism). Kleiber (1947) used metabolic data ranging from the mouse (0.02 kg) to the cow (600 kg) and obtained the exponent 0.756. The results of Brody and Proctor (1932) and Kleiber (1947) had correlation coefficients of at least 0.95 and were significantly different from 2/3 but not from each other. Kleiber suggested that since taking the exponent as 3/4 led to a mathematical simplification in calculation (which were done by log tables at that time), it was preferable to use 3/4 as the value of the exponent.

It is noteworthy that most of the interest centred on interspecific as compared to intraspecific calculations. Kleiber (1947) noted that when the data set contains a narrow range of masses, it is more difficult to distinguish between exponents with statistical confidence. He pointed out that for several sets of intraspecific data there was a wide variation of exponents, most of which could not be distinguished from either 3/4 or 2/3. He proposed that, until more evidence was available, the most rational hypothesis would be to assume that the interspecific and intraspecific exponents were the same. Kleiber also noted that in order to distinguish between coefficients of 1.0 and 0.75, data is required from animals differing in mass by at least a factor of three. Thus, for our purposes, where the extrapolation is from mice to humans (rather than from shrews to elephants), the differences between 2/3 and 3/4 as exponents may in some cases, depending on the uncertainties in the data, be irrelevant.

Allometric relationships for a large number of physiological parameters have been reviewed and summarized by numerous authors including Peters (1983), Calder (1981), Gunther and Guerra (1957), Spells (1969), Gould (1966) and Adolph (1949). Table 13 lists the coefficients (b) and exponents (k) for some physiological parameters which have been reported by some investigators to be related to mass, M, or weight, W, by the relation

$$y = bM^k. \tag{24}$$

In general, the reported exponents tended (since Kleiber's and Brody's work) to cluster about three numbers: 1.0 (for various volumes such as tidal volume, blood volume, and lung capacity); 0.75 (for energy metabolism rate, minute volume, blood flow, cardiac output, clearance and oxygen uptake); and -0.25 (for pulse rates, breath rates and inverse lifetimes). These numbers stimulated various investigators to develop a theoretical basis for allometric relationships. These developments will be discussed in Section 4.2.3. In addition, and more importantly for our purposes, these numbers have played a prominent role in the development of various pharmacokinetic models for the behaviour of drugs in humans and other mammals.

The allometric equations for time-periods such as breath time, heartbeat time and lifespan aroused considerable interest in that time seemed to play a special role. These and other time-periods scale roughly as $M^{0.25}$ (i.e. the

TABLE 13

Various physiological parameters as functions of body mass[a] (Gunther, 1975)

Function/organ	Coefficient (b)	Exponent (k)
Organ masses[b]		
Adrenals	0.3	0.80
Brain	10	0.70
Heart	5.8	0.98
Kidneys	7.3	0.85
Lungs	11.3	0.99
Cardiovascular		
Cardiac output (ml/min)	166	0.79
Heart rate (min^{-1})	236	−0.25
Stroke volume (ml)	0.66	1.05
Blood flow (g/s)	3.3×10^{-3}	0.74
Respiratory		
Respiratory freq (s^{-1})	5.5	−0.28
Tidal volume (cm^3)	6.3×10^{-3}	1.00

[a] Note the exponents and coefficients will vary somewhat from one author to another.

[b] Organ weights in grams.

inverse of rates which scale as $M^{-0.25}$). This scaling was consistent with the idea of "physiological time" which was first discussed by Brody (1937). He pointed out that the growth curves for a number of animals, ranging from the albino mouse to the cow, could be put on the same curve by plotting per cent of mature weight against the product kt, where k is the growth rate and t is the time after puberty. This plot was the first use of a rescaled time axis. (We will see more examples of this method in Section 5.) There was a major exception in the early part of the curve for humans because of neoteny, the phenomenon of prolonged early development. Neoteny (Gould, 1979; Boxenbaum and D'Souza, 1990) is the retention of shapes and growth rates which characterized the juvenile stages of the human ancestors, leading to a relatively (compared to other mammals) later development of mature features and relativcly longer lifespan.

Brody (1937) proposed that the relationships of growth curves and the higher (per unit mass) rates of metabolism of smaller versus larger animals supports the idea of a physiological time for animals based on body mass whereby 1 day in the life of a mouse has the same significance as 50 days in the life of an elephant. This concept has been considerably expanded by Boxenbaum (1982b) and will be discussed in Section 5.2.5.

The concept of physiological time was further developed by Gunther and Guerra (1955) who used the analogy of a pacemaker or "biological clock" which determines the rhythm of each function such as heart rate, durations of sexual cycle, respiratory rate, and lifcspan. They also noted that, because breath time and heartbeat time have essentially the same exponents, the ratios of the exponents are nearly independent of mass, which leads to the conclusion that there are four heartbeats per breath. Similarly, Gould (1979) concluded that mammals have approximately 800 million heartbeats in a lifetime, except humans, who have about three times that number, which corresponds to the relatively longer lifetime in humans, attributable to neoteny.

Work by Kleiber (1932) and Brody and Proctor (1932) let to the value of 0.75 for the exponent in the allometric equation for energy metabolism and provided the foundation for a considerable body of work. The value of 0.75 for the allometric exponent was virtually unchallenged until 1982, when Heusner (1982a) argued that Kleiber, Brody and others had assumed in their analysis that the coefficient, b, in the allometric equation, $y = bM^k$, was the same constant for all species. He questioned the validity of this assumption which is equivalent, in his view, to assuming that every mammal of unit mass (where $y = b$) has the same metabolism in spite of gross anatomical differences. Heusner proposed that the mass coefficient, b, should be allowed to vary between species in order to account for diffcrences between species. He used an analysis of covariance model which lead to the equation (for basal metabolism rate)

$$y = bM^{0.67}, \tag{25}$$

where b depends on the species. Thus, Heusner essentially arrived at the Meeh equation. Interestingly, when Heusner did a regression analysis assuming b to be a constant, he obtained for his set of data (which was a compilation of data from several workers) a result very similar to Kleiber, with the exponent equal to 0.776, which was significantly different ($P < 0.0005$) from 0.75. He proposed the use of 2/3 as the exponent for all species and obtained values of the mass coefficient, b, using this assumption.

Thus, it would appear that we have come full circle back to the surface law. However, Feldman and McMahon (1983) pointed out that there was a monotonic increase in Heusner's mass coefficients as a function of mass. Using the same data and a reparamctrization of the statistical model used by Heusner, they showed that the mass coefficient, b, varied as mass to the power 0.08. Thus, Heusner's model

$$y = bM^{2/3}, \tag{26}$$

where b depends on the species (and therefore on mass) combined with the result (Feldman and McMahon, 1983)

$$b \propto M^{3/4-2/3} \tag{27}$$

gives exactly the same result as

$$y = bM^{3/4}, \tag{28}$$

where b is a constant over all species. Feldman and McMahon (1983) also reported that the 95% confidence intervals put the exponent for intraspecific (within the same species, for example from children to adults) scaling between 0.612 and 0.728, whereas for interspecific (across species) scaling it is between 0.744 and 0.760. Thus, according to Feldman and McMahon (1983), it would appear that we have the "surface law" (eq. 25) for intraspecific scaling and the Kleiber Law (eq. 27) for interspecific scaling.

Travis and White (1988) have recently argued that Heusner and McMahon are, in fact, in agreement that the correct value of the exponent is 3/4. They go on to state that the value of 3/4 " is now well accepted as the appropriate scaling for metabolic rates." This interpretation is, however, not supported by the literature (Chappell, 1989).

Wieser (1984) agreed with the analysis by Feldman and McMahon and attempted to provide a rationale for their results. He proposed that one of the primary problems had been a confusion between the ontogeny and phylogeny of metabolism. He pointed out that much of the early work on energy metabolism which supported the surface area law had involved intraspecies comparisons (e.g. Rubner, 1883), whereas Kleiber (1932, 1947) had used interspecies as well as intraspecies data. Wieser proposed, then, that the ontogeny of metabolism should not be confused with the phylogeny of metabolism. He asserted, as we noted in Section 4.1, that the rate of metabolism of a mammal changes in a complex manner during its development through various life stages. In each of these stages, the allometric exponent for metabolism is different from the previous or following stage. For example, he asserts that the exponent for a growing fetus is similar to a maternal organ, whereas after birth there is a rapid increase. Most of the lifetime of a mammal is spent in the mature stage which Wieser (1984) calls Phase 4. In that stage, he proposes the surface law exponent, 2/3, applies. But he says that the validity of the surface law for intraspecific relations in adults does not imply that the same exponent would result upon comparison of adults of different species. Heusner (1982b) also proposed a somewhat similar framework for ontogeny but he distinguished only three phases: (1) a phase of growth with structural changes accompanied by a significant mass increase and a mass exponent greater than 2/3; (2) a stable adult phase where there may be some mass changes but the organism remains geometrically similar with a mass exponent of 2/3; and (3) a senescent phase where the mass exponent is less than 2/3.

Wieser (1984) also proposed that the phylogenetic aspects of metabolism are responsible for the fact that the mass coefficient in the intraspecific relationship varies from one species to another in a systematic manner, as noted by Feldman and McMahon (1983). This variation of the mass coefficient with the mass is, as shown by Wieser (1984), proportional to the mass to the power 0.09, which is very similar to the result of Feldman and McMahon, and thus yields an interspecific relationship with an exponent of 0.75.

More recently, Hayssen and Lacy (1985) have reviewed the work of several investigators on the allometric relationship for basal metabolism rates. They noted that a common weakness in the interspecific comparisons by various investigators was the use of multiple data points for the same species; for example, they point out that Kleiber (1947) used six values for domestic rabbits, four for dogs, two for sheep, and three for women as a part of his 26 data points. They argue that this confounds the intraspecific and interspecific trends and violates the assumption of statistical independence of the samples. The concern of these two investigators (Hayssen and Lacy) is the apparent elevation of the Kleiber equation (eq. 28) to the status of a biological law; thus, for example, they feel that the difference between Kleiber's exponent of 0.75 and Brody's of 0.73 is important. For the same reason, they feel that the use of domestic populations for much of the data is not appropriate. With these concerns, they developed a massive data set from the literature, consisting of 293 species from 14 mammalian orders. They use the mass-specific basal metabolic rate (BMR), i.e. the BMR divided by the mass, as the independent variable. They performed a least-squares regression and found that for a linear fit the exponent was -0.30 and was significantly different from Kleiber's result of -0.25 (resulting from dividing by mass). But they also found that many of the species deviate significantly from their model; for example, 21% of the species have BMRs more than 50% above or below the predicted values. They concluded that no single equation adequately describes the allometric relation between body mass and BMR for mammals. They went on to comment that the Kleiber–Brody law had become so imbued with "an aura of authenticity over time that researchers have failed to modify and improve on it as more data have become available." This is particularly ironic since the same criticism was made by Kleiber (1932) of the surface law.

Most of the actual regression analyses have been performed on log-transformed data (e.g. Brody, 1947). Very few authors have questioned this approach. Hayssen and Lacy (1985) acknowledge the possibility of other

choices by the statement that "the data were log-transformed first in order that the residuals from least squares regressions would more closely approximate normal distributions". They did not, however, indicate whether this was an assumption or whether they had confirmed this behaviour. The question of whether to log-transform or not was raised in 1964 by McWilliams *et al.* and again in 1968 by Zar. These authors noted that there is not simply one way to fit a curve to data, and that the choice of curve-fitting method will affect the result. McWilliams *et al.* (1964) discuss three models which all involve minimizing the sums of squares of deviations: (1) using untransformed and unweighted data; (2) using unweighted log-transformed data (the method traditionally used in allometry); (3) involving the use of log-transformed, statistically-weighted data (not mentioned by Zar). If the statement by Hayssen and Lacy (1985) regarding the distribution of residues is correct, then the second and usual approach is correct. It is not clear that this assumption has been checked against the data used by various authors.

One of the specific concerns of Hayssen and Lacy, however, is the previous use of domesticated animals such as laboratory rats, dogs, etc., because of the artificial diets—most of these animals have been bred for rapid growth on nearly unlimited diets. Thus, Hayssen and Lacy exclude data from such species in their calculation. On the other hand, they use human data which would seem subject to the same objection. Certainly, one concern about the data from "wild" species would be the uncertainty due to having less control over and smaller numbers of experimental animals. But perhaps more germane to our concerns is that they excluded exactly those animals of greatest concern to this review, namely the comparison between humans and those laboratory animals commonly used for toxicological and pharmacological experiments.

Thus, in spite of 150 years of effort, there is still a controversy about whether there is an allometric relationship for energy metabolism (the quantity which began the debate) and, if so, what the exponent is. The preponderance of evidence indicates that such a relationship exists and that the exponent for energy metabolism and other similar parameters is not equal to 1 and is somewhere close to 0.7. In view of the inherent variations and uncertainties, rarely mentioned, in measuring basal energy metabolism or even mass (for large animals), it seems somewhat unimportant for practical applications whether the value of the exponent is 0.67, 0.7 or 0.75. Table 14 illustrates the difference in the mass ratios to the powers of 0.67, 0.7, 0.75 and 1.0 of a 70 kg human and a mouse (0.02 kg), a mature rat (0.4 kg) and a dog (10 kg). Note that for the exponents 0.67, 0.7 and 0.75 there is almost a 100% diffcrence for the mouse, a 50% differenee for the rat, and a 20% difference for the dog. Davidson *et al.* (1986) discuss these

TABLE 14

The values of the mass ratio (Q) of a 70 kg human to a 0.02 kg mouse, a 0.4 kg rat, and a 10 kg dog, to the powers 0.67, 0.7, 0.75 and 1.0

Animal	$Q^{1.0}$	$Q^{0.75}$	$Q^{0.7}$	$Q^{0.67}$
Mouse	3500	455	302	237
Rat	175	48.1	37	31.8
Dog	7	4.3	3.9	3.7

results and summarize some of the important issues and complications in a "principle" as follows.

PRINCIPLE. Intraspecies extrapolations are associated with less intrinsic error than interspecies extrapolations. Numerical scaling of biological data from laboratory animals to man most often does not take into consideration important factors such as age and gender, polymorphism, diet, environment, state of health, or route of chemical administration and scheduling. The two most commonly used bases of extrapolation, $W^{1.0}$ and $W^{0.67}$, for scaling biologic data of laboratory animals (mouse, rat) to man, provide scaling factors and hence human equivalent values differing by approximately 10-fold ($W^{1.0}$ and $W^{0.67}$). In contrast, scaling factors derived from bases $W^{0.67}$ ("surface area") to $W^{0.75}$ ("basal metabolic rate"), indusive, differ by less than 2-fold, an extent of variance well within the probable error associated with any single one of these bases. The scaling factor derived from any body weight base is particularly susceptible to error due to the variances of body weight among laboratory animals, and therefore, the choice of body weight used for extrapolation must be justified from experimental study data.

COROLLARY. When a human equivalent dose is quoted as extrapolated from laboratory animal data, the allometric base for the extrapolation and the body weights used for the human and for the laboratory animal should be defined and specified and, in the case of the body weight of the laboratory animal, justified from experimental study data.

4.2.3 Theoretical Basis

In his essay "On Being the Right Size", Haldane (1928) points out that "the most obvious differences between different animals arc differences in size, but for some reason zoologists have paid singularly little attention to them". Clearly, the allometric relationships, both interspecific and intraspecific,

that involve a wide range of physiological parameters illustrate that size, as measured by mass, plays a central biological role. The reason for this role is certainly far from understood, but some lines of inquiry have been explored. From an intraspecific viewpoint, one might guess that the basic mechanisms of growth are important. From an interspecific viewpoint, it would be natural to propose that such relationships are the result of natural selection or are the result of constraints upon evolution related to, for example, physical laws governing the transport of energy and mass- or load-bearing by limbs.

The reasoning of Sarrus and Rameaux (1938) regarding heat transfer across surfaces is one of the possible explanations for allometric relationships. While the surface law has had a resurgence of interest in recent years (Heusner, 1982a; Feldman and McMahon, 1983; Wieser, 1984), it does not have the acceptance that existed before 1932. Nevertheless, there are certainly physical constraints on the heat production and heat loss of animals, particularly homeotherms that involve transfer of heat across surfaces. We noted that there are numerous ways (Gould, 1966) in which an organism can compensate for the decrease of surface area to volume with increasing mass. One such method involves developing complex structures to increase surface area (e.g. brain and lung).

Huxley (1932) was probably the first person to introduce the allometric equation in the context of growth within a species. He noted that if a single animal were followed in time from birth, its various organs and parts of the body would tend to increase with mass according to the power law or allometric equation. Huxley also noticed that if the quantity of intensity (y) and the mass (M) were considered to be functions of time (t), and assuming

$$y = bM^k, \tag{29}$$

then taking the log of both sides and differentiating with respect to t (assuming b is independent of time), the following is obtained

$$y^{-1}\left(\frac{dy}{dt}\right) = kM^{-1}\left(\frac{dM}{dt}\right). \tag{30}$$

In this equation, the relative rate of growth of y (whatever it is) is proportional to the relative rate of growth of mass.

Huxley deduced this equation and the allometric equation by assuming that growth is the process of self-multiplication and that the growth rate is affected by the age and the environment in a general way. He thus proposed that the rate of growth of any organ is proportional to a constant specific to that organ, the size of that organ at that time, and a general factor, G, which depends on age and environment and is the same for all organs. These

assumptions result in the equation (Huxley, 1932)

$$\frac{dx}{dt} = axG, \tag{31}$$

for organ x (a is a proportionality constant). He also assumed that a similar equation held for the rest of the body which he designated y leading to

$$\frac{dy}{dt} = cyG. \tag{32}$$

where c is a proportionality constant. Solving for G in each equation and setting the expressions for G equal to each other results in

$$\frac{dy}{dx} = \frac{cy}{ax}, \tag{33}$$

or

$$y = bx^k, \tag{34}$$

where $k = c/a$. One weakness in this approach is that the use of eq. (32) for the "rest" of the body assumes the overall constant b, which is the weighted average of the growth constants for the rest of the body, is independent of y and of time.

Huxley (1932) suggested that the factor G was probably a simple function of the difference of the size of the organism from its final size at any given time. Note that eq. (30) shows that k is a measure of the relative growth rates of a given organ and the whole body. Huxley proposed that k be called the growth-coefficient of the organ. If $k \neq 1$, the organ is growing at a different rate from the body and is said to be heterogonic. If $k = 1$, the organ is said to be isogonic.

Huxley (1932) then demonstrated several examples of the growth formula in different species. He also discussed the use of allometry in other branches of biology such as taxonomy, comparative physiology, genetics, and phylogeny. Bertalanffy (1957) proposed that there are essentially three metabolic types of animals, depending on whether $k = 1$, $k > 1$, or $k < 1$, and that these three types also have different growth characteristics in the sense of Huxley.

Gould (1966) discussed the role of allometry in ontogeny and phylogeny. His goal was to assess the role of absolute mass as a cause of trends in the alteration of form. (In this regard he notes that he does not mean to imply "that size increase is the efficient cause of shape alteration. In phylogeny that role is, of course, played by natural selection ... size increase does become a formal cause of adaptation in determining a specific direction in

which natural selection must operate... .") He suggested two types of size-correlated shape changes. Some shape changes are mechanically required by an increase in size. Other trends may not be causally related but nevertheless are implicd by size change.

The first case requires a form change with size increase while the second permits "the expression of new potentiality." Natural selection then becomes the factor which produces adaptation.

Gould gives several examples of size-required shape changes. These include the problem noted by Galileo (1637). As mentioned earlier, Galileo argued that the diameters of supporting structures of bodies have to increase at a disproportionate rate as their size increases. This is because the breaking strengths of supporting structures are proportional to their cross-sectional area. As a result, he argued that the diameters of supporting limbs must increase at a rate closer to the half power of mass than to the third power of mass, thereby "changing their shape until the form and appearance ... suggest a monstrosity." Otherwise, an animal weighing 100 times as much as a similar shaped animal would be supported by limbs that have a cross-sectional area only 22 times larger than the smaller animal. These changes may be limiting in terms of size increases; for example, the above increase in leg diameter would eventually lead to a leg width greater than body length (which would increase as the 0.33 power of the body mass). The stress put on limbs by dynamic loading may be a more important determinant of size than the static loading.

Gould also suggests that increasing metabolism per unit mass with decreasing size results in a lower limit to mammalian size of about 2.5 g (the size of a small shrew); however, Schmidt-Nielsen (1984) suggests that it is the increase in heartbeat frequency with decreasing size that is the limiting factor (the heartbeat of a 3 g shrew is about 1030 per minute at rest).

Gould (1966) noted that while the parameters in allometric equations probably do not reflect directly efficient physiological process, they may directly reflect causes in terms of adaptation. In this sense, adequate adaptation in new situations and sizes, i.e. natural selection, becomes the final cause of change.

Gould (1966) also noted that heat flux and its relationship to surface area is not the only physiological function that relates shape to size. Functions such as locomotion, respiration and the delivery of required nutrients also have important effects in the shape and size of limbs, lungs, the circulatory system and the stomach. These different functions could lead to exponential coefficients which differ from the surface rule exponent of 2/3.

The allometric equation appears to give a good empirical description of the changes in such physiological parameters as heartbeat frequency and blood vessel diameter with size increases resulting from ontogeny and phylogeny. Various authors have argued for other mathematical relationships, such as a linear function or a power series. Aside from the lack of data needed to validate more complex models, Gould (1966) suggests that there are three criteria to be used in deciding between various possible mathematical expressions: (1) adequate statistical fit; (2) simplicity of form and (3) ease of interpretation. The allometric equation, $y = bx^a$, satisfies these criteria better than any other to date.

Its lack of simplicity may be one reason that an equation proposed by Economos (1977) never attracted much attention. Economos proposed that the discrepancy of energy metabolism from the surface area law was due to gravity. Clearly the effect of gravity was the cause of the previously discussed observation by Galileo. Economos noted that hypergravity research has shown that small mammals have a larger gravitational tolerance than large mammals. He proposed that there is a "metabolic cost of gravity" which is the energy that land mammals would have to continuously expend to maintain posture and form. Economos argued that the form of the energy equation was the sum of a term representing the metabolic cost of gravity and a term independent of gravity and which followed the surface area law. Using data on the gravitational tolerance of five species (mouse, rat, chicken, dog and man) and assumptions about the relationship of "gravitational metabolic cost" to gravitational tolerance, Economos (1977) proposed the following equation for energy metabolism, E

$$E = 58M^{0.67} + 12M^{0.89}. \tag{35}$$

He then used metabolic data on 27 land mammals and found a slightly better fit (a smaller standard error; statistical significance was not mentioned) with his equation than with Kleiber's equation.

While arguments about growth and natural selection have not yet resulted in a rigorous derivation of the allometric equation, they do provide plausibility and potential constraints on the exponent. Clearly an increase in overall size, while maintaining essential biological functions, does lead to increases in the sizes of various physiological parameters. There are very reasonable arguments for not increasing all parts of the body in proportion to total mass. The transport of heat across the surface area of an organism is an important process, but not the only one, that couples physiological function to size. Moreover, as noted earlier, the lack of homogeneity would lead us to expect that the exponent would differ from 2/3 and probably vary from one species to another, even if heat transfer were the governing mechanism. Such arguments, of course, only state that various physiological parameters are a function of mass, but they don't give that function. Huxley's argument gives plausibility, but not proof. Indeed, with all the complicated factors involved in maintaining a biological organism and in

natural selection, it would be a surprise if the relationship between various physiological parameters and body mass were as simple as $y = bM^k$. But, this could be the most important (largest) term in the "true" expression. Certainly, there is a large body of empirical data to support this view, even though there are still arguments about the value of k when y stands for energy metabolism.

4.2.4 *Allometry and Biological Similarity*

The progression of organisms to larger sizes either through growth or natural selection can be viewed, in some ways, as a scaling process. Such a process is familiar to engineers, who before building a full scale system, will first test out the system at smaller sizes (bench scale, pilot plant, etc.). A very useful approach for predicting the appropriate dimensions at various sizes is the theory of similarity, which is also called dimensional analysis. There are various kinds of similarity, depending upon the processes involved in the system. These processes or functions might be geometrical, mechanical, hydrodynamic, biological or combinations of the above.

A very extensive review of the application of dimensional analysis to biological systems was written by Gunther (1975). He pointed out that the rules of geometrical similarity have been known since Euclid's time (300 B.C.). Archimedes (287–212 B.C.) later established that, for "similar" geometrical shapes, the corresponding surfaces increase as the square and the volumes increase as the cube of the linear dimensions. It was this result, in fact, that was used in deriving the exponent of two-thirds which appears in the surface law (eq. 6).

As previously mentioned, Galileo (1637) introduced dynamic aspects of similarity into his discussion of the scaling of animals. He argued that neither terrestrial animals nor trees can grow beyond a certain size because a change in relative proportion, as well as harder and stronger materials, are required as size increases.

Several other well-known scientists such as Newton played important roles in the development of dimensional analysis or similarity analysis. It was Maxwell who introduced the idea of dimensional analysis. The basic idea is that the fundamental physical laws underlying the interaction of matter and energy in all its forms can be expressed in terms of a very few dimensions, namely mass, length, time and temperature. All valid equations expressing relationships between various physical and biological parameters must have consistent dimensions. This limitation puts severe restrictions on the forms of the relationships (although it does not give unique

relationships). An example is the ideal pendulum. The only physical information available is the mass, m, length, l, and the acceleration of gravity, g. The dimensions of g are L/T^2 where L is a length and T is time. We can use dimensional analysis to derive the period of a pendulum by noting that the dimension of period is T. Assume that the period t, is given by

$$t = m^a g^b l^c \text{ or}$$
$$T = M^a L^b T^{-2b} L^c. \tag{36}$$

m, g and l being written in terms of their dimensions in the latter. Clearly $a = 0$, $c = -b$, and $b = -1/2$ so that one possible answer is

$$t = \left(\frac{l}{g}\right)^{1/2}. \tag{37}$$

Of course, $t = k(l/g)^{1/2}$, where k is a constant, will also have the right dimensions so we have not completely determined the period, but we have severely restricted its form assuming it has the form given in eq. (36). Indeed, the correct result is given by $t = (2\pi)(l/g)^{1/2}$.

We could go further and ask how we would have to change the pendulum length if we increased its mass to obtain the same period, and the answer is trivial: not at all. But the ideal pendulum is well-known to be one of the simplest models in physics; other models involve a much more complex analysis. How does this example apply to the issue at hand? We are concerned with changing from one organism to another in such a way as to retain the same physiological activities interacting in the same manner. A constant efficiency of interacting biological activities with an increase in size implies systematic, size-correlated shape alterations (Gunther, 1975).

When scaling from one system to another it is necessary to specify the criteria that will be used to determine whether the two systems are the same. Gunther (1975) proposes four kinds of physical (as opposed to geometric) similarity: dynamic, kinematic, hydrodynamic and thermal. Dynamical similarity exists if homologous parts of the systems experience similar net forces. Kinematic similarity relates to motions (homologous points at homologous times). Hydrodynamic similarity involves having the same ratio of inertial to viscous forces. Thermal similarity implies similar temperature distributions and heat-flow at homologous surfaces. The consequences of electrodynamic similarity have been discussed in another paper by Gunther and Guerra (1955).

Following Gunther (1975), let us define any variable Q which is a power function of mass, length, and time

$$Q_1 = (M_1^a)(L_1^b)(T_1^c). \tag{38}$$

For the scaled-up version of the system, we can define the same variable as

$$Q_2 = (M_2^a)(L_2^b)(T_2^c), \tag{39}$$

where M_1, L_1, T_1 and M_2, L_2, T_2 are the corresponding masses, lengths and times of the two systems. Now a quantitative relationship can be established by defining Newton's reduction coefficient

$$x = \frac{Q_1}{Q_2}. \tag{40}$$

If $M_1/M_2 = m$, $L_1/L_2 = l$, and $T_1/T_2 = t$, then

$$x = m^a l^b t^c. \tag{41}$$

Once we determine a, b and c we can obtain the ratio x. But first we can reduce the number of variables by deciding which of the similarity criteria apply to our situation; for example, in dynamic similarity (sometimes called mechanical similarity), we assume the mass density and the acceleration of gravity are constants. Since mass is the ratio of volumes and since $g = L/T^2$ is a constant, we see that $m = l^3$ (because mass is equal to the product of a density, assumed constant, and a volume which has dimensions of length, L, cubed) and $t = l^{1/2}$ (because g = constant implies that L is proportional to T^2), so that

$$x = l^{3a+b+0.5c}. \tag{42}$$

In kinematic similarity, mass density is a constant and $l = t$ giving

$$x = l^{3a+b+c}, \tag{43}$$

whereas in hydrodynamic similarity, the Reynold's number and the kinematic viscosity are constants, giving

$$x = l^{3a+b+2c}; \tag{44}$$

and in electrodynamic similarity, Gunther and Guerra (1955) obtained

$$x = l^{2a+b+c}. \tag{45}$$

Specifying the reduction coefficient in terms of the length ratios is not as directly related to the issue at hand as the use of mass. Since for all cases $m = l^3$, we can rewrite eqs. (42–45) in terms of M and thereby generate allometric equations involving powers of the mass. Table 15 shows the exponents that will appear for various quantities under the criteria of dynamic and kinematic similarity.

Lambert and Tessier (1927) proposed that biological similarity should have the same relationships as kinematic similarity. This implies, for example, that blood velocity and density are independent of size, criteria

TABLE 15

Calculation of reduced exponents for dynamic (D) and kinematic (K) similarity[a]

	Dimensions			Similarity reduced exponent	
	M	L	T		
	a	b	c	D	K
Acceleration	0	1	−2	0.00	−1.00
Area	0	2	0	0.67	0.67
Energy	1	2	−2	1.33	1.00
Force	1	1	−2	1.00	0.67
Frequency	0	0	−1	−0.16	−0.33
Length	0	1	0	0.33	0.30
Power	1	2	−3	1.16	0.67
Speed	0	1	−1	0.16	0.00
Viscosity	1	−1	−1	0.50	0.33

[a] Adapted from Gunther (1975) and Gunther and Guerra (1955).

that are reasonably well satisfied. Notice that this choice leads to the surface law for power (which has the same units as basal energy metabolism) rather than Kleiber's law. Heusner (1982a,b) has also advocated that choice.

Kinematic or biological similarity will not yield the appropriate value of the exponent according to Kleiber, namely 0.75. In order to account for this difference as well as differences between predicted heartbeat frequency (exponent of −0.33) and experimental heartbeat frequency (exponent of −0.25), Gunther and Martinoya (1968) introduced an empirical correction. This empirical correction is required only when time is involved in the variable of interest. Gunther and Martinoya replaced eq. (43) with

$$x = m^k m^{0.065T}, \tag{46}$$

where

$$k = a + (b + c)/3 \tag{47}$$

and we converted from l to m using $m = l^3$. In their empirical correction, Gunther and Martinoya (1968) stated that $T = 0$ if no clock is actually used in the experiment. But when a clock is used (e.g. to determine frequency or period) then $T = 1$ for periods and $T = -1$ for frequencies. Thus, for heartbeat frequency $T = -1$, whereas for heartbeat period $T = 1$. For

TABLE 16

Comparison of results using operational time factor[a]

Entity	T	Experimental exponent	Theoretical exponent
Area	0	0.62 to 0.67	0.67
Compliance	0	0.82 to 1.00	1.00
Energy	0	0.97 to 1.06	1.00
Power (metabolism)	−1	0.73 to 0.75	0.73
Frequency	−1	−0.25 to −0.27	−0.27
Period	1	0.25 to 0.27	0.27
Volume	0	0.99	1.00
Volume flow	−1	0.76	0.74

[a] Adapted from Gunther and Martinoya (1968).

pressure, a measurement that can be directly made without a clock, $T = 0$, even though $c = -2$. Table 16 shows the result of this empirical correction and the comparison of the theoretical reduced coefficient and the empirically determined coefficient.

Gunther (1975) discusses the application of the theory of kinematic or biological similarity to a wide range of functions and shows that when the concept of operational time is included, most functions are well-described. In fact, a correlation coefficient of 0.99 is obtained between calculated and empirical values for the exponential coefficient.

McMahon (1973) proposed still another criterion for similarity analysis. He assumed that elastic stability criteria were the important determining factors. These criteria relate to buckling and bending forces; buckling is the phenomenon whereby a lateral displacement of a vertical structure causes the weight to apply a toppling moment which overcomes the elastic forces; bending forces are the forces that cause a horizontal structure (e.g. a tree limb) to droop. When applied to structures, these criteria lead to relationships between length and diameter whereby the length is proportional to the two-thirds power of the diameter. McMahon argued that the same phenomena operate on a quadruped. When standing still, the four limbs are subject to buckling loads while the vertebral column is subject to bending loads. When running, there are bending loads on the limbs and buckling loads on the vertebral column. Thus, he argued that if l is the length and d is the diameter, then under elastic criteria l^3 is proportional to d^2. Furthermore, since the volume, and therefore the mass, is proportional to the product of l and d^2, both will be proportional to l^4. Since the surface area

is proportional to ld (neglecting the ends of the cylinders), the surface area will be the 5/8 the power of the mass. This value is very close to the mass exponents of 0.63 and 0.65 found by Hemmingsen (1960) and Stahl (1967), respectively.

McMahon then derived an expression for energy metabolism by assuming that the tensile strength of muscles and their shortening velocity are constants from one species to another. This assumption is based on experimental data obtained by Hill (1950), and it leads to the conclusion that the power output of any particular muscle and, therefore, all metabolic variables involved in maintaining energy flow to the muscle, depend on the cross-sectional area of the muscle, which is proportional to d^2. Since d is proportional to mass to the power 3/8, the muscle power, P, is given by

$$P = b(M^{3/8})^2 = bM^{0.75}, \tag{48}$$

which is the Kleiber result.

McMahon points out that since lung volume is proportional to M and alveolar volume to $M^{3/4}$ (according to this theory) then respiratory frequency is proportional to $M^{-0.25}$. A similar argument holds for ventricular stroke volume, cardiac output, and heart rate.

Thus, by applying elastic similarity criteria and making two important assumptions about muscular function, McMahon was able to derive the Kleiber result. McMahon (1984) has compared empirical data on several species, including primates and ungulates, and obtained very large correlation coefficients for fits of linear dimensions versus body weight to his theoretical equations, giving length proportional to $M^{0.25}$ and diameter proportional to $M^{0.375}$.

Blum (1977) pointed out that while the ratio of the areas of two geometrically similar three-dimensional objects is the two-thirds power of the ratio of their masses, the ratio of the area of two four-dimensional objects is the three-fourths power of their mass ratio. He suggested that perhaps the surface rule does apply, but to a four-dimensional system. He suggested one possible candidate for the fourth dimension would be the "minimization of the ratio of the free energy cost of ion pumping per unit area to the free energy cost of assembling/maintaining the metabolic machinery per unit volume". A similar suggestion was made more recently by Yates and Kugler (1986), who proposed that an additional thermodynamic-related dimension is necessary in allometric scaling to account for complex processes such as transport and excretion of chemicals.

Derome (1977) noted that the most general development of similarity analysis is in terms of group theory. He discussed the group theory implications of the allometric equations and suggested that there could be

situations where a generalized allometric equation involving the product of powers of two quantities (e.g. mass and length) would give a better representation of the data, particularly, he suggested, when comparing two different series of animals. We will discuss something similar to this idea in Section 5.2.5 where body weight and brain weight are used as surrogates.

More recently, Heusner (1982b) has revived the earlier work of Lambert and Tessier (1927) and noted that their application of biological similarity (which is the same as the kinematic similarity of Gunther, 1975) agrees with his results (discussed in the previous section). He presents a long argument involving the dimensions of the mass coefficient, which he contends proves that the exponent for energy metabolism is equal to two-thirds; however, these arguments rely on the assumption that the ratio of homologous lengths and times are equal.

Gunther (1975) argues that in all likelihood no single similarity rule applies to all living organisms because of their complexity. It is clear that the basic processes involved in life include mechanical, hydrodynamic, electrodynamic and thermodynamic phenomena. As a result he suggested that there is a mixture of these regimes. In this view, it seems unlikely that similarity analysis can ever arrive at a unique value for the exponent in the allometric equation.

4.2.5 *Physiological Constants*

Stahl (1963) applied similarity analysis in a slightly different way from that just described. He looked for ratios and products of parameters that were independent of size or body mass. This was based on a mathematical theorem proposed by Buckingham (1914): a physical system which can be described by a set of dimensional parameters can also be equally well described by a smaller set of non-dimensional parameters. These non-dimensional quantities are called physiological constants or invariants; they contain information about the criteria involved and are useful without a complete understanding of the governing physical principles. This approach is empirical but is useful in situations where the phenomena are too complex (for example the design of artifical legs) to develop the governing equations.

Stahl took all of the known variables and attempted to combine them in such a way as to yield quantities that were invariant under size changes. One such example (Stahl, 1963) is the product of the minute volume of volumetric flow of air, G_v, and the breath time, T, divided by the equivalent lung volume, V (which is estimated at one-third the lung mass). These quantities are empirically related to mass through the allometric equations

shown below:

$$G_v = 120M^{0.74}, \tag{49}$$

$$T = 4.7 \times 10^{-5} M^{0.28}, \tag{50}$$

$$V = 1.24 \times 10^{-2} M^{0.99}. \tag{51}$$

We see that

$$G_v T/V = 0.45 M^{0.03}. \tag{52}$$

Thus, the above quantity $(G_v T/V)$ is nearly independent of mass. Indeed the exponent 0.03 is not significantly different from zero; that is, it is within the statistical errors in the exponents of the allometric eqs (49)–(51).

Stahl (1963) compiled a large list of such invariant numbers. He defined the exponent of the mass in such numbers as the residual mass exponent (RME); for example, the RME in eq. (52) is 0.03. Clearly the RME should be a small number if the ratio is to be nearly independent of mass (and, therefore, species). Stahl (1963) compiled 48 such numbers with RME values of 0.03 or less. These include such ratios as the volume of red cells to whole blood volume, the mass of total haemoglobin to the lung mass, the breath time to the pulse time, renal diodrast clearance to water intake, and production of carbon dioxide over the use of oxygen. These "biological invariants" provide another approach to interspecies extrapolation. One such application was made by Boddington (1978), who noted that for most species the product of the basal metabolism rate and the longevity divided by the body mass is nearly independent of mass, i.e. a physiological constant. As a result, the longer the lifetime the slower the metabolic rate per unit mass. Boddington suggested that this result implies the existence of a finite total metabolism, which if used at a faster rate, results in a shorter lifetime.

4.3 APPLICATION TO THE SCALING OF TOXICITY DATA

In principle, the application of the allometric methods to the extrapolation of toxicity data involves choosing the appropriate dependent variable y and obtaining the best fit of the allometric equation to the data. The problem is that while the independent variable in the equation $y = bx^k$ is the body mass $(x = M)$, the dependent variable may not be easily interpreted (e.g. acetylcholinesterase inhibition). The dependent variable might be the LD_{10} (the lowest dose needed to kill 10% of the animals), the MTD or the NOAEL (the no observed adverse effect level). Moreover, there may be cases, as we will see in Section 5.2.5, where a multivariate allometric equation is required.

Krasovskii (1976) described an extensive programme carried out at the Sysin Institute in Moscow where, apparently, a large number of allometric equations were developed for physiological parameters ranging from liver enzyme activities to various cardiovascular parameters. Unfortunately, these data are not given in the paper. Krasovskii also described the application to toxicity data and compared the results with those obtained by the surface area method and by extrapolation from the most sensitive species; the results were given in summary form for approximately 100 substances. He did not list the substances nor give the data for the toxicity parameters. He found that, on the average, the allometric method and surface area method gave fairly similar answers. He reported that regression analysis was applicable to 80–85% of the substances and that the predicted values differed from actual values by a factor of less than 3 or 4.

Book (1982) applied the allometric method to the scaling of nitrogen dioxide (NO_2) toxicity data. He began with data for concentration and exposure times required to kill 50% of the exposed animals, for five laboratory species (mouse, rat, guinea-pig, rabbit, and dog). He then developed an allometric equation for the 1 min dose (calculated from the concentration multiplied by the minute volume). The dose (millilitres per minute) was proportional to body mass to the power 0.84. This result predicted that the LD_{50} for humans weighing 70 kg would be 4.9 ml.

Book noted that for the five animal species, the lethal concentration was proportional to a power of the time of exposure and that the exponents were fairly constant across the species, with no apparent dependence on body mass. He then used the mean of the exponents (−0.28) to develop a similar equation for 70 kg humans. This equation predicted that for humans "the concentration of NO_2 for a 60-minute exposure to fifty percent lethality would be 174 ppm NO_2". This value is close to the value of 150 ppm proposed as a lower bound for lethality from NO_2 for an unspecified exposure period [National Academy of Sciences, Committee on Medical and Biological Effects of Environmental Pollutants (NAS), 1977] and to the value of 150 ppm for 1–2 h exposures (Shy, 1973).

Mordenti (1986a) analysed the data used by Freireich *et al.* (1966) in their classic study (discussed in Section 3.3) in which they used the surface area law to bring consistency to toxicity data (LD_{10}, MTD) for chemotherapeutic drugs. Of the 18 drugs used by Freireich *et al.* there were sufficient data on 14 of them (Table 17) to perform regression analyses, and statistically significant results were obtained for 10. Table 18 gives the exponents (for the equation $D = aM^b$, where D is dose in milligrams) and significance level obtained by regression analysis on data for the 14 drugs. As can be seen from Table 18, the exponents varied between 0.6 and 0.87. While the surface area result of 0.67 falls in the range of the data, the data do not support the choice

TABLE 17

A comparison of small animal LD_{10}s and large animal (including humans) maximum tolerated doses (mg/kg)[a]

Agent	LD_{10} Swiss mouse	BDF mouse	LD_{10} rat	MTD dog	MTD human
Actinomycin D	0.07	0.12	0.09	0.03	0.015
BCNU[b]	11	16	6.6	2.4	2.5
Busulphan	15	15	3.7	6.0	0.7
Cyclophosphamide	93	110	12	12	10
5-Fluorouracil	42	45	25	10	15
5-FUDR	160	190	89	40	30
Mechlorethamine	1.3	0.9	0.37	0.48	0.2
Melphalan	6.3	9.7	—	1.5	0.9
6-Mercaptopurine	86	62	51	22	27
Methotrexate	3.3	5.2	0.58	0.12	0.41
Mitomycin C	2.3	2.2	1.3	—	0.2
Nitromin	45	31	7.1	4.4	2.0
L-Phenylalanine	5.1	5.5	2.3	0.63	0.2
thio-TEPA	5.7	6.5	2.7	1.1	0.2

[a] Adapted from Freireich *et al.* (1966).
[b] BCNU = 1.3-Bis(2-chloroethyl)-1-nitrosourea.
[c] FUDR = 2-Fluoro-2'-deoxyuridine.

of 0.67 over other values such as 0.75; it does explain, however, why Freireich *et al.* found a good correlation with the body surface area.

In a recent article, Travis and White (1988) repeated the analysis of the Freireich *et al.* data and also performed the same analysis of the data of Schein *et al.* (1970). They obtained results similar to those of Mordenti, but they argue that these results support the exponent of 0.75 for the allometric equation, whereas Mordenti proposes an empirical approach without supporting any particular value for the exponent.

Clearly, similar calculations could be done for other databases. Unfortunately, the data on chemicals other than chemotherapeutic drugs is generally inconsistent and non-uniform compared with the chemotherapeutic drug data. However, a few examples for Mo and Ba are given in Section 3. The data for Mo is too variable to use, but when linear regression analysis is applied to the data on $BaCl_2$ in Table 9, with assumptions about the animals' masses corresponding to Table 4 (using

TABLE 18

Allometric exponents for the toxic dose (mg) of 14 drugs used by Freireich *et al.* (1966)

Agent	Exponent	Significance level[b]
Actinomycin D	0.776	0.011
BCNU[c]	0.784	0.005
Busulphan	0.727	0.053
Cyclophosphamide	0.765	0.117
5-Fluorouracil	0.841	0.015
5-FUDR[d]	0.782	0.001
Mechlorethamine	0.815	0.054
Melphalan	0.721	0.004
6-Mercaptopurine	0.868	0.034
Methotrexate	0.704	0.145
Mitomycin C	0.707	0.0005
Nitromin	0.663	0.007
L-Phenylalanine	0.602	0.0005
thio-TEPA	0.621	0.001

[a] From Mordenti (1986a). Note that the original data (Table 17) were given in units of mg/kg and the above results are for the total dose in mg.
[b] Determined by *t*-test.
[c] BCNU = 1,3-Bis(2-chloroethyl)-1-nitrosourea.
[d] FUDR = 2-Fluoro-2'-deoxyuridine.

0.1 kg for the rat), the result is an exponent of 0.7 with a correlation coefficient of 0.98.

These results indicate that the use of regression analysis to develop allometric equations relating equivalent doses to a power of the body mass is better than an arbitrary number if adequate data exist.

4.4 DISCUSSION AND CONCLUSIONS

In an entertaining paper, Kleiber (1967) pointed out that when the Lilliputians calculated the required dietary intake for Gulliver, they arrived at a daily allowance of 1728 Lilliputian daily portions (this example first appeared in Thompson, 1917). Kleiber noted that this value would result from an allometric equation with the exponent of 0.76. He went on to note that had the Lilliputians used the surface law they would have given Gulliver only 675 portions and he would probably have starved. Of course, Kleiber

was biased. It is conceivable that obesity might have eventually resulted from the 1728-portion diet.

As mentioned in the beginning of Section 4, the "surface law", that is, relationships to mass to the power 0.67 in the case of metabolic rates, for example, or 0.33 in the case of time periods, is an allometric relationship. Thus, allometry as a general approach neither supports nor denies the "surface law" concept. The main contribution made by Huxley and Tessier (1936) and others was to provide a more empirical approach to intraspecies and interspecies extrapolation, which does not *a priori* assume a value for the exponent in the allometric equation. This more empirical approach led, quite naturally, to the use of linear regression analysis to obtain the value of the exponent from the data. Using this technique, Kleiber (1932) and Brody and Proctor (1932) reported that this best fit to the energy metabolism data was given by a value close to 0.75 rather than 0.67.

As noted by Kleiber (1947), over a small range of masses the two exponents do not make very much difference to the final result. The difference between the two exponents in extrapolating from a 10 kg dog to a 70 kg human is only 20%, whereas in extrapolating from a 20 g mouse to a 70 kg human, the difference is a factor of 2. (The choice of the mouse and dog are representive of the maximum range of mass used in toxicity experiments to obtain data for risk assessment extrapolations to humans.)

At least two important consequences have resulted from the development of the allometric approach. One is a more theoretical development; namely, that the fact that the exponents in allometric equations were not always those that would arise from the surface law led to alternative proposals to explain the value of 0.75. These proposals ranged from dimensional analysis arguments regarding elastic stability (McMahon, 1973) to the introduction of a fourth dimension (Blum, 1977). The other important consequence was that investigators were no longer tied to a single value for an exponent to which they tried to fit all the data. The idea that the coefficients and exponents should be obtained experimentally became widely accepted. With this acceptance came the recognition that many physiological variables obeyed an allometric equation.

The many allometric relationships tying several quite different species together in terms of body mass are probably not accidental and are most probably the result of natural selection. Gould (1966) in his review gives an elegant presentation on the relationship of allometric equations to ontogeny and phylogeny. For the purposes of extrapolating toxicity data to humans, the considerable success of many investigators provides a note of optimism. There are indeed similarities between species which allow for the extrapolation of at least some physiological data from one animal to another. The scaling is likely to be as a power of the mass (although a

multivariate approach involving brain mass or other parameters may sometimes be needed). Whether the power is 0.67 with some adjustments to the coefficient, as argued by Heusner (1982a), or 0.75 or some other value, is not important unless there are no data that would give empirical values for the coefficient and exponent in the allometric equation. If there are no such data, then the choice of 0.67 is more conservative (i.e. gives a lower value for the safe dose) than either 0.75 or 1.0.

The argument about the value of 0.75 versus 0.67 as the correct allometric exponent continues. In a recent article, Travis and White (1988) argue that the correct value is 0.75. Contrary to their assertion that this value has been well-established, we would argue that the arguments involving similarity provide certain constraints but no rigorous proof of any particular value for the exponent. Clearly the need for heat balance, elastic similarity and other fundamental requirements must be met simultaneously. The solution to all these constraints was arrived at differently by different species, so that it would be surprising if the same exponent held for all physiological parameters. Nevertheless, these similarity requirements constrained the adaptations over time within some range of values for the allometric exponent.

We propose that it is misleading to use values such as 0.67 or 0.75 because the presence of two significant figures implies an accuracy which is not supported by data or theory, and in the past has tended to lead to neglecting contrary data. A somewhat similar point was made by Davidson *et al.* (1968) who noted that the difference between using exponents of 0.75 and 0.67 is "well within the probable error associated with any single one of these bases." We believe the best approach is to develop the allometric equation empirically, using the best available data. In the absence of adequate data to perform a regression analysis, we recommend (Chappell, 1989) the use of 0.7 as the allometric exponent because it gives a conservative result, differing little from that obtained by using 0.67 or 0.75, but without the appearance of an accuracy which doesn't exist.

The note of optimism mentioned above has to be balanced by the considerable lack of adequate data for developing allometric equations, as well as the difficulty in choosing and interpreting the dependent variable y in the allometric equation $y = bM^k$. Moreover, there are probably many cases where allometry didn't work, and these failures were not reported. The toxicity literature reveals very little consistency in the conduct of experiments. Much work needs to be repeated in order to obtain data that can be compared between species. Even the development of acute and subchronic data, which are less expensive than chronic experiments, would test the validity of allometric scaling, and, until better data are obtained, the exponents given by short-term experiments could perhaps be used for extrapolating chronic data to humans.

This use of acute data to help extrapolate chronic results presumes that similar mechanisms are operating at very different dose levels. The weakness of the allometric approach is that it is fundamentally empirical and does not give an understanding of underlying mechanisms that are involved, such as clearance. In Section 5, we will see that this connection with mechanisms can be made by introducing pharmacokinetic concepts. The use of these concepts then explains the dose scaling in terms of clearance rates that scale as some power of the mass, frequently in the range of 0.6 to 0.8. Clearance can be shown to be proportional to a volume of distribution (which generally is proportional to the first power of the body mass) divided by a biological half-life (which, as we have seen, is generally proportional to mass to the power 0.2–0.4).

5 Pharmacokinetics and Comparative Metabolism

5.1 INTRODUCTION

The approaches taken in Sections 3 and 4 have largely ignored mechanisms involved in the toxic or therapeutic actions of chemicals in that they take a strictly empirical approach to the relationship between equivalent doses in different species. In recent years, particularly in the last decade, there has been a considerable interest in applying the principles of pharmacokinetics to the topic of interspecies scaling. While most of this work has been focussed on pharmaceutical drugs, the basic principles should be equally applicable to the toxicological actions of chemicals whether they are organic or inorganic. This work has been fairly successful (NAS, 1987) in elucidating those important underlying mechanisms that give the scientific basis for using allometric equations for interspecies scaling. Of particular interest is the fact that short-term studies of absorption, distribution, metabolism and excretion (ADME) of chemicals in laboratory animals can be used to determine whether allometric scaling is justified and to determine the exponents to use in such scaling. The pharmacokinetic approach focusses on the concepts of absorption, biological half-life, plasma concentration of the chemical, plasma clearance, distribution volumes, metabolism and excretion.

On the other hand, numerous investigators have emphasized the differences between laboratory animals and humans and between animals of the same species. Brodie (1962) pointed out that different inbred strains of rats oxidize antipyrine at widely different rates (as much as a factor of 3). He also noted that humans vary greatly (as much as an order of magnitude) from one person to another in their rate of metabolism of drugs. Calabrese (1986) has also emphasized the human heterogeneity in the susceptibility to

toxic agents. There is also great species-to-species variability in metabolic pathways not only in the relative importance of various pathways (e.g. deamination of amphetamine is a major route of metabolism in rabbits, but is almost absent in rats and dogs; Brodie, 1962) but also the presence or absence of pathways (dogs cannot acetylate primary amines; Brodie, 1962). Thus, there are reasons to suspect that, at least in some cases, pharmacokinetic models will not be useful in extrapolation, particularly if they are too detailed.

In spite of the many differences, considerable progress has been made in bringing together pharmacological data from different species into a single framework. This progress was made by the application of the allometric approach to pharmacokinetic data. In Section 5.3 we review this progress, which began when several investigators realized that what seemed like major interspecies differences in biological half-lives and equilibrium tissue levels of chemicals were actually related by means of allometric equations. These parameters were interrelated by means of clearances which themselves appeared to follow allometric equations [a phenomenon noted by Adolph (1949) who found the exponent for inulin clearance to be 0.77].

With the advent of modern analytical equipment, it was found that while the equivalent doses (for the same response) varied greatly between different animals, there was considerable agreement between total or unbound (depending on the degree of binding) plasma concentration of a chemical, and that the same concentration in different animals often led to the same response. The plasma concentration can, in turn, be related to the clearance, the volume of distribution and the dose (for linear pharmacokinetics). If clearance and volume of distribution obey an allometric equation, then the concentration also obeys an allometric equation (Mordenti, 1985b).

For chronic dosing, the average plasma concentration is equal to the amount absorbed from the gastrointestinal tract into the systemic blood (including effects of first pass metabolism) multiplied by the dose rate (e.g. milligrams per day) divided by the clearance. Mathematically, the clearance is proportional to the volume of distribution (which is the volume of the tissue through which a given amount of the chemical would have to be distributed to give the measured concentration) of the drug divided by the half-life. Thus, response, dose, clearance, volume of distribution and biological half-life are mathematically interrelated. If clearance obeys an allometric equation with the exponent of 0.67, when the surface law can be used to scale between species. If the exponent is 0.75, the Kleiber–Brody approach gives a more accurate estimate in interspecies scaling.

Linear pharmacokinetic models show that the dose divided by area under the concentration versus time curve, for a single dose, is equal to the clearance. This gives a way of obtaining the clearance from a single-dose experiment which can then be used to obtain the allometric scaling for chronic exposure.

Dedrick *et al.* (1970) used methotrexate to show that a simple rescaling of dose and time brought the concentration versus time curves for five species, including humans, onto one curve. This approach has been extended by Boxenbaum (1982b), who calls the rescaled time "pharmacokinetic time"; it is the same concept as "physiological" and "biological" time which was discussed in Section 4.2. While this time frequently scales with an exponent between 0.2 and 0.4, the exponent is not the same for each chemical. Furthermore, Boxenbaum has shown that for some chemicals, particularly those involving phase I hepatic metabolism, brain mass as well as body mass must be used in the allometric equation.

Section 5.3 discusses two pharmacokinetic models for interspecies scaling. One is the allometric model, which is based on the classical pharmacokinetic multi-compartmental approach (generally no more than three compartments) and involves parameters such as half-life, clearance, volumes of distribution and area under the concentration–time curve. In this model, the individual pharmacokinetic parameters are determined for several species. Linear regression analysis is performed to obtain the allometric equations. The equations are then solved for humans (i.e. $M = 70$ kg).

The other model is the PB-PK model, which involves a detailed mass balance around the important compartments (liver, kidney, etc.) using differential equations. There are several coefficients involving flow rates, metabolic rates, and other anatomical, physiological, thermodynamic and transport properties. These coefficients are determined from existing data, experiments or, in some cases, allometric equations. The proponents of these models claim that they can account for non-linearities (e.g. saturation of metabolic pathways) and for active metabolites. The disadvantage is that they are expensive, time-consuming, and are so complex that they require considerable experience in order to avoid misinterpretations. These two approaches, the PB-PK and allometry paradigms, have been compared and contrasted in a recent chapter by Boxenbaum and D'Souza (1990).

5.2 LITERATURE REVIEW

5.2.1 Biological Half-life, Plasma Concentration and Allometry

While animal physiologists during the first half of the century focussed on correlations between species, the pharmacologists were devoting

considerable attention to divergences between species. As recently as 1958, Quinn *et al.* published a report of their work on species, strain and sex differences in the metabolism of hexobarbitone, aminopyrine, antipyrine and aniline. One of their principal findings was that there were significant species differences in rates of metabolism. The half-lives of hexobarbitone, antipyrine and aniline varied significantly among the species (mouse, rat, guinea-pig, rabbit, dog and human—the human data actually came from another study), with the half-life for a mouse being the shortest (19 min) and that for a human the longest (360 min).

Quinn *et al.* (1958) also studied the activity of the liver microsomal enzyme, which inactivates hexobarbitone, and found it to be inversely related to the duration of activity or "sleeping time" in the species studied. The duration of activity also increased with biological half-life. They concluded that because of the species variation, the design of new drugs was complicated because the metabolic rates in humans could not be predicted from animal data. They did suggest, however, that since the number of enzyme systems responsible for drug metabolism are relatively few in number (this statement is less true now with the discovery of the large diversity of isoenzymes), it might be possible to use structural and physical properties to predict the fate of a drug. However, because of the differences between species and strains, they believed such predictions would only be valid for an inbred strain.

It is of interest to note that Quinn *et al.* pointed out that "mice metabolized these drugs much more rapidly than the other animals". Although drug metabolism and energy metabolism are not necessarily related, this behaviour is consistent with the concept of physiological time introduced in Section 4.2. The application of linear regression analysis for the hexobarbitone data of Quinn *et al.* (1958) does not yield a statistically significant result; however, the analysis of their results for the demethylation of aminopyrine does yield an allometric equation for the relative enzyme activity (mg/g/h) with an exponent of -0.27 (negative because of division by body weight) and a correlation coefficient of -0.96 which is significant at the 0.99 level.

In 1962 and 1963 there was a series of papers by Richmond *et al.* (1962a, b) and Furchner and Richmond (1963) describing studies in which radioactively labelled doses of Zn, Cs and I were administered to different species. The animals were then followed to determine biological half-lives, retention and excretion. The results consistently showed that the biological half-lives were an increasing function of mass, and that the biological (discounting radioactive decay) equilibrium level (body burden) of the elements used obeyed an allometric equation with an exponent of about 0.4.

In 1966 Fujita *et al.* did a similar study using radioactively labelled Cs and K. They found that the equilibrium body burden to intake ratio for a given dose obeyed an allometric equation involving body mass with exponents of about 0.45. Fujita *et al.* (1966) proposed that the reason for this scaling was that renal clearance obeyed a surface area law involving not total body mass, M, but rather kidney mass, M_k. That is,

$$\mathrm{CL} = aM_k^{2/3}. \tag{53}$$

They made this assumption on the basis of limited measurements of renal clearance.

Clearance is conceptualized as the body fluid volume per unit time that is completely cleared of whatever substance is of interest. If the fluid of interest is plasma and the clearance is by urinary excretion, then

$$U = P\mathrm{CL}, \tag{54}$$

where U is the urinary excretion (grams per day), P is the plasma concentration of the chemical in question (Cs or K) (grams per millilitre) and CL is the plasma clearance (millilitres per day).

Fujita *et al.* (1966) then used other data to obtain an allometric equation relating kidney mass to body mass with an exponent of 0.84, which is very close to the exponent of 0.85 reported by Adolph (1949). With the assumption of a steady state where the excretion is equal to the input and proportional to the urinary excretion u, they showed that the ratio of body burden, Q, to intake, I, at equilibrium, is

$$A = \frac{Q}{I} = \frac{kf_u MB}{P\mathrm{CL}}, \tag{55}$$

where k is a constant, f_u is the ratio of urinary to total excretion, B is the mean body concentration and M is the whole body mass.

As can be seen, eq. (55) contains the ratio of urinary excretion to total excretion and the ratio of mean body concentration of the nuclide to the plasma concentration of the nuclide. Fujita *et al.* (1966) examined both these ratios and found them to be species invariants, that is, independent of mass in the animals (rabbits, humans and rats) for which they had data. They found that f_u was approximately 0.8–0.85 for both Cs and K for the various species and that B/P was approximately 10 for both Cs and K. Thus, the only parameters in eq. (55) which depend on body mass are M and CL. Using eq. (53) and

$$m_k = 0.029M^{0.84}, \tag{56}$$

which is very close to the result obtained by Adolph (1949)

$$m_k = 0.0212M^{0.85}, \tag{57}$$

Fujita *et al.* obtained

$$A = K_1 M^{0.44}, \tag{58}$$

where k_1 depends on f_a, K and B/P.

The weakest part of the argument is eq. (53) because Fujita *et al.* do not present very convincing data to support it empirically and because their theoretical arguments are identical to those used since Sarrus and Rameaux (1838) to relate physiological parameters to whole body mass. But the idea that clearance obeys an allometric equation is consistent with the results of Adolph (1949) who found that inulin clearance varies as body mass to the power 0.77.

Fujita *et al.* (1966) then showed that, assuming the simple exponential function model (or one compartment model), the biological half-life $t_{1/2}$ is given by

$$t_{1/2} = 0.693 \frac{A}{f_1}, \tag{59}$$

where f_1 is the fraction of the nuclide absorbed from the gastrointestinal tract into the blood. This result implies that biological half-life obeys an allometric equation and is an increasing function of mass. They also reported that other data in the literature supported allometric equations for the half-lives of Na and water with exponents of between 0.16 and 0.25. They suggested that since Na and water are reabsorbed at the renal tubules, it is reasonable that these should behave differently from K which is secreted at the tubules.

This paper by Fujita *et al.* was one of the first to bring pharmacokinetic parameters such as clearance and biological half-life together with allometry in an attempt to use fundamental processes in interspecies scaling. A key element in their derivation was the assumption that body burden was proportional to plasma concentration.

5.2.2 *Plasma Concentrations and Responses*

In 1967 Brodie and Reid proposed that many of the problems involved with relating the effects of drugs on laboratory animals to the effects on humans could be resolved by comparing plasma concentrations of the drugs. They noted that the reluctance to do so arose from previous difficulties with assay procedures, which had been overcome in the 1940s and 1950s. They also felt that the work of Freireich *et al.* (1966) relating toxic concentrations of chemotherapeutic drugs to surface area had contributed to the feeling that plasma concentrations were not needed.

Brodie and Reid argued that the response to a drug is determined by the amount fixed to the drug receptors and stated "Since the drug-receptor complex is generally in dynamic equilibrium with the unbound drug in the plasma, the effects of the drug will be related to the plasma level." They pointed out, however, that in cases where the drug metabolites are the active agent, the response will probably be related to the plasma concentration of the active metabolite(s). On the other hand, they noted that the picture may be quite different for drugs which act non-reversibly. For such drugs, which are first attached reversibly to the sites of action and then react chemically with the receptors, the drug will remain attached to these sites long after the rest of the drug has been cleared from the body. Many such drugs are rapidly metabolized. As a result, their biological effect (toxic or therapeutic) will not be related to the plasma concentration at any given time but rather to the initial concentration. These drugs will usually cause more intense responses with high concentrations of short duration than with low concentrations of long duration.

Brodie and Reid also pointed out that the biological half-life or duration of action has an important effect on the differences seen between laboratory animals and humans in drug response. The fact that biological half-lives of chemicals tend to be much longer in humans than in laboratory animals had led to many mistaken conclusions in pharmaceutical testing; for example, they noted that a screening programme designed to find a barbiturate that was rapidly metabolized in the body led to a thiobarbiturate that was the longest lasting barbiturate in humans because of the failure to account for differences in biological half-lives. Chloramphenicol, which was thought to cause a special disease that killed newborn children, was actually causing death from overdose because it was metabolized so slowly by human children compared with laboratory animals.

By taking into account both duration of action and plasma concentration, Brodie and Reid were able to show that many examples of seemingly different responses of humans and laboratory animals to various drugs were actually examples of similar responses; for example, although the duration of action of a particular tranquillizer varied in four species, from 0.1 h in the mouse to 10 h in the cat, the plasma levels were almost identical on recovery of the righting reflex. Brodie and Reid (1967) pointed out that the duration of action of the drug was three times longer in female rats than in males, although the plasma level of each at recovery was similar. This result is consistent with the work of Quinn *et al.* (1958) who observed that female rats metabolized hexobarbitone about four times more slowly than males. This gender-dependence illustrates the need for the use of safety factors. In another example of the relationship between plasma concentration and response, Brodie and Reid noted that sodium retention by phenylbutazone

can be produced in rats by daily doses of 400 mg/kg and in humans by daily doses of 5–10 mg/kg, but the plasma concentrations in both species are quite similar.

Brodie and Reid also pointed out that the therapeutic effects of some drugs that were discovered accidentally in humans have been missed in animal screening. One such example is the anti-inflammatory effect of phenylbutazone. Preliminary screening in animals showed a negligible action because the half-life in small animals is 3–6 h compared with 3 days in humans.

Brodie and Reid also made recommendations for the use of plasma concentration and biological half-life in clinical practice. They suggested that initial studies be performed where single small intravenous dosages are given. The measurement of plasma concentrations at frequent intervals would then give data that would allow for the determination of the biological half-life from the slope of the logarithm of the concentration–time curve. The extrapolation of the curve back to time zero would give the initial concentration, and from this the volume of distribution, V, is obtained (in the single compartment model) as follows:

$$V = \frac{D}{C(0)}, \tag{60}$$

where D is the dose and $C(0)$ is the initial concentration.

Multicompartment models are more complex than (60) because there is more than one volume of distribution involved. The distribution volume is conceptualized as the effective volume of tissue over which the drug is distributed. Given V, the amount of drug remaining in the body at any given time is the product of V and the plasma concentration at that time.

Brodie and Reid (1967) went on to describe a procedure for determining dosages and dosage intervals that would ensure that plasma concentrations would fluctuate by no more than 50%; thus allowing for maintenance of quantities of the drug that are above the therapeutic and below the toxic concentrations. This was one of the first papers to suggest that the dosage interval should be proportional to the biological half-life of the chemical.

The work by Brodie and Reid (1967) was important because they were among the first to propose *as a general principle* that for many chemicals the plasma concentration of the unbound chemical is correlated with the biological response. Furthermore, they emphasized the importance of the biological half-life in interpreting data from animal screening tests and devising therapeutic schedules. It is interesting that they did not suggest that the work by Freireich *et al.* (1966) gave a way of relating dosage to plasma concentration. Instead, they felt that the antitumour agents that were involved in the study by Freireich *et al.* had a different mode of action than

"usual medicinal agents" and were, therefore, unique in their behaviour. Of course, Crawford *et al.* (1950) had already shown that when doses of sulphadiazine for humans of different sizes were equivalent on a surface area basis the blood levels were also similar.

One of the earliest reports that did relate plasma concentrations to dose was by Wagner *et al.* in 1965. In this paper, they reported the results of an analysis of pharmacokinetic compartment models using an analogue computer. They found (the result can also be derived mathematically) that the average asymptotic plasma concentration was

$$C_{av} = \frac{FD}{VKT}, \tag{61}$$

where F is the fraction of dose absorbed, D is the dose given at the beginning of each dose interval, V is the apparent volume of distribution of the chemical, K is the first-order rate constant for overall loss of the chemical from the blood, and T is the length of the dose interval. C_{av} is defined as

$$C_{av} = T^{-1}\int_{t_1}^{t_2} C_1(t)\,dt, \tag{62}$$

where $t_2 - t_1 = T$ and C_1 is the plasma concentration at equilibrium. The integral is the area under the time–concentration curve from t_1 to t_2.

Wagner *et al.* (1965) pointed out that eq. (61) will hold only if the following conditions are true:

(a) Transfer from the blood is first order.
(b) F, D, V, K and T are constants for each dose in a given subject.
(c) The dynamics of input, transport, and excretion are described by a system of simultaneous linear differential equations.

Under these conditions it is possible to show that following a single dose:

$$FD = VK\int C(t)\,dt = VK(\mathrm{AUC}). \tag{63}$$

The integral in eq. (63) is the area under the curve of the concentration – plot, denoted as AUC below. It follows from the assumptions that

$$C_{av} = \frac{F(\mathrm{AUC})}{T}. \tag{64}$$

Furthermore, although this wasn't mentioned by Wagner *et al.* (1965), if the total clearance from the plasma is CL, then

$$\mathrm{CL} = \frac{FD}{\mathrm{AUC}} \tag{65}$$

and therefore

$$C_{av} = \frac{FD}{TCL}. \quad (66)$$

If the dose is given intravenously, then $F = 1$.

These results have several important consequences. One is that single-dose experiments can be used to determine the average concentration for multiple-dose or chronic studies. A comparison of AUC for i.v. and oral administered single doses will give the value of F. This is a more reliable method than comparing amounts in urine and faeces, because it is not possible to distinguish biliary excretion from non-absorption in the gastrointestinal tract. The equation also clearly shows that (for one-compartment models) C_{av} is proportional to the biological half-life as follows:

$$t_{1/2} = \frac{0.693}{K}. \quad (67)$$

Moreover, as Wagner *et al.* (1965) note, the total asymptotic accumulation is given by

$$V C_{av} = \frac{1.44FDt_{1/2}}{T}. \quad (68)$$

There are many situations where the conditions required for eq (61) to be valid are not met. Wagner *et al.* listed several of these, including the following.

(a) Plasma level exceeds protein binding capacity (K will increase or V decrease at high plasma levels).
(b) The enzyme system metabolizing the chemical is saturated (no longer first order elimination).
(c) The fraction of drug absorbed decreases as dose is raised (F will decrease as D increases).
(d) The drug's metabolism is inhibited by another agent (K decreases).

Most of these situations probably occur only for doses much greater than those of interest for deriving a NOAEL.

Several factors can affect clearance and/or biological half-life. These include age, health status and the presence of other pollutants. Renal impairment (Salvadeo *et al.*, 1988) has been shown to lead to as much as a 10-fold increase in the biological half-life of ibopamine. The area under the concentration–time curve (AUC) showed similar effects, with the AUC for those with severe renal impairment being 20 times that for normals. Even those described as having a mild degree of renal impairment in this study had an average biological half-life for ibopamine that was twice that in the normal group.

Age also has the effect of increasing biological half-life and the AUC. Robertson *et al.* (1988) found a 75% increase in half-life and a 100% increase in AUC for orally administered nifedipine in elderly volunteers (average age of 77.8 years) compared with younger volunteers (average age of 27.1 years). They also found bioavailability increased (by 33%) in the elderly group. Scott *et al.* (1988) also found increased half-lives (approximately 65%) and AUC (by 30%) in elderly compared with younger humans.

It is clear that decreased CL and increased AUC is equivalent to a longer half-life and a higher concentration in the blood for a given dose. As a result, if the calculation is made for a young, healthy human, a safety factor needs to be included, when necessary, to protect those who are ill and/or older.

In 1969, Mellet discussed a wide range of issues involved in comparative drug metabolism. The importance of metabolism is clearly seen from eqs (61), (65) and (67), which involve rate constants, clearance and biological half-lives. Mellet's review covered a number of issues concerning differences and similarities between species and various factors influencing drug metabolism. These include age (e.g. both newborn and elderly animals or humans can respond abnormally), disease and nutrition status (starvation can increase half-lives), multiple-drug effects (other chemicals can stimulate or inhibit enzymatic activity and therefore change CL or $t_{1/2}$, sex (female animals are more sensitive than males to a number of drugs; some of these effects can be reversed by giving male and female hormones to the opposite sex) and strain (different rates of metabolism for some drugs).

Given that there are many differences, nevertheless, many of the reactions that occur in laboratory animals also occur in humans. Mellet noted that while exceptions occur, the main differences lie in the rates of metabolism and excretion. He discussed in great detail the different classes of metabolic conversion: oxidations, reductions, hydrolyses and conjugations. While noting that there are many species differences (e.g. carnivorcs convert aniline differently from herbivores), he emphasized that the search to find a laboratory animal that metabolizes drugs in a qualitatively similar way to humans is doomed to failure and that the existence of numerous alternative pathways for metabolism tends to obliterate species' differences and results in the rate being more important than the specific pathway (with some exceptions, for example the conversion to an active metabolite in some species and not in others). Mellet (1969) then went on to discuss the relationship of drug metabolism rates, plasma concentrations and pharmacological response to size. Drawing upon the work of Pinkel (1958) and others (discussed in Sections 3 and 4), he noted that if one assumes, following Brodie and Reid (1967) and others, that

pharmacological response is correlated with the plasma concentration of the chemical, then the allometric equations developed by Adolph (1949) and others, which relate blood volume, cardiac output, renal function and other physiological parameters to a power of the body mass, are relevant to plasma concentration, excretion rates and other pharmacokinetic parameters. For example, greater cardiac output per kilogram of body weight will lead to a more rapid flow to the liver in a monkey (which turns over its entire blood volume in 20 s) than in a human (where the turnover time is 50 s). If blood flow to the liver is the limiting factor in the metabolism of a chemical, then the clearance in a monkey will be greater than in a human.

Mellet described the results of his experiments administering single doses of cyclophosphamide to various species (mouse, hamster, dog, rat, monkey, and human) to calculate the AUC of the concentration–time curve. For single doses, the large differences in biological half-life (from 17 min for mice to 200 min for humans) make direct comparison of concentrations difficult. By way of illustration, if we assume a mono-exponential decay curve (i.e. single-compartment model, which is rarely the case), the concentration is given by the following:

$$C(t) = C(0) \exp(-kt), \tag{69}$$

where k and $t_{1/2}$ are related to each other as in eq. (67) and $C(0)$ is equal to the dose divided by the apparent distribution volume [see eq. (60)].

However, for single doses it could be argued that it is not any single value of $C(t)$ that is correlated with the response (except for some non-reversibly acting chemicals), but some combination of C and the duration of action. This suggests that AUC, as defined in eq. (63), is a more appropriate parameter to equate. Mellet (1969) gave various doses and took blood samples in order to obtain $C(t)$. He then attempted to relate AUC for the same dose for the different species and found that when the dose was expressed in milligrams per kilogram, correlation between AUC for animals and humans was very poor. When the dose was expressed in milligrams per square metre of surface area (using the same approach as Freireich *et al.* (1966)), surface area equivalent doses gave very nearly the same value for the area under the curve for all species. Mellet also plotted the AUC versus $Dt_{1/2}/M$ and found that there was a very good correlation between these parameters, as would be expected, since using eq. (69) gives

$$\mathrm{AUC} = \frac{Dt_{1/2}}{0.693V}. \tag{70}$$

For many compounds, the volume of distribution, V, is proportional to M.

If the AUC obeys an allometric equation for interspecies scaling (as Mellet's data suggests), then the average plasma concentration will also obey an allometric equation as seen from eq. (66). If the exponent of the allometric equation for the AUC is approximately 2/3, as Mellet's data suggest, and if we use eq. (64), we would find that multiple dosing at intervals of T will give an average concentration whose dependence of D, M and T is given by

$$C_{av} = \frac{aD}{TM^{2/3}}, \tag{71}$$

where a is a constant and D/T is the dose rate (milligrams per day). This result, assuming that the response is proportional to the average plasma concentration, would explain the previous results of Freireich *et al.* (1966) and others discussed in Section 3. It implies that surface area equivalent doses in chronic dosing lead to the same plasma concentrations, which in turn lead to the same (therapeutic or toxicological) biological responses. However, as we have seen, there are many physiological parameters for which the allometric exponent is not 2/3. If a rule of thumb were desired, an exponent of 3/4 or 0.7 might be better. But, where possible, empirical data on these parameters for different species should be used to determine an appropriate value for the scaling exponent. This value can be expected to be in the range of 0.6–0.8 (with some exceptions).

Mellet (1969) reviewed a number of other experiments which show that biological half-life increases with body size, but he never did propose a relationship. The use of eq. (70) with the assumption that V is proportional to M (which is the case for many distribution volumes), would lead to the result

$$T_{1/2} = bM^{x}, \tag{72}$$

where x would generally be in the range 0.2–0.4. This result is related to the concept of "physiological time" which was discussed in Section 4.2 (as seen in Table 14 by taking time as inverse frequency). A recent article by Davidson *et al.* (1986) mistakenly quotes Mellet as giving the exponent in eq. (72) as 0.67.

Mellet (1969) concluded "that for many drugs there is a valid relationship among species between dose, plasma levels, body size, and responsc". He recommends that when attempting to determine toxic doses for humans LD_{10} is determined in small animals and MTD in large animals. Drug disposition studies and plasma concentrations should be studied after administering doses (parenterally) at the LD_{10}, half the LD_{10}, and one-tenth the LD_{10}. Then the AUC for the doses should be determined. If the AUCs are proportional to the dose in milligrams per square metre or to milligrams per kilogram $t_{1/2}$ for different laboratory animals, the proportionality is

likely to hold for humans. If these relationships do not hold, then Mellet proposed that it is unlikely that a prediction can be made. Mellet discussed factors,virtually the same as those mentioned by Wagner *et al.* (1965), that could invalidate these relationships.

Swabb and Bonner (1983) were the first investigators to publish *a priori* predictions of serum concentration–time curves using the allometric approach described above. They used pharmacokinetic data from mice, rats, rabbits and two species of monkeys for the drug aztreonam. They designed the human clinical trials for this drug by scaling clearance and volume of distribution using a one-compartment model and, in spite of the fact that this drug displays two-compartment behaviour in humans, made remarkably good predictions of its behaviour in humans. Mordenti (1985b) reported the successful scale-up of pharmacokinetic data for ceftizoxime from animals to humans based on the data from mice, rats, monkeys and dogs. In this case a two-compartment model was used.

In 1986 Collins *et al.* reported that the use of AUC data to determine the starting doses for human testing of chemotherapeutic drugs would, in many cases, be more efficient than using the surface area approach. They evaluated the pharmacokinetic data from humans and mice for several anticancer drugs and concluded that the use of AUC gave a better prediction of the maximum tolerated dose than the technique developed by Freireich *et al.* (1966) that was described in Section 3.3.

Davidson *et al.* (1986) have proposed a third principle (the first two have been quoted earlier) summarizing some of the material we have just discussed which reads as follows:

PRINCIPLE. The allometric equation

$$\text{dose (for a given toxicity index)} = aW^n$$

adequately describes quantitative interspecies relationships for acute toxic responses to most xenobiotics (80 to 85%). The power exponent, n, is often observed to be 0.67 (e.g., anticancer agents), and, for the majority of compounds, varies between 0.62 and 0.8, although for a significant number of compounds or toxic endpoints some other exponent may be obtained, including 0 and 1.0. For any given xenobiotic, the probability of the best extrapolation prediction from laboratory animals to man is provided by scaling on the base W^n (where n is determined experimentally from four to six species), less well by $W^{0.67}$, and least well by $W^{1.0}$. For any given xenobiotic, the kinetics of body disposition and, in particular, metabolism, are important factors intrinsic to interspecies allometric correlation of toxicologic parameters. Although species differences in pharmacokinetics and metabolism are known to exist for some xenobiotics, in general the kinetic parameters of half-life ($t_{1/2}$), area under plasma concentration-time curve (AUC), clearance (CL), and hepatic enzyme activity, including P-450 monooxygenase system, correlate with interspecies body weight with powers of 0.65 to 0.75. [Note—Davidson *et al.* presumably meant to say that the half-life scales with an exponent of 0.25–0.35.]

COROLLARY. Extrapolation predictions from laboratory animals to humans based on regression analyses of toxicologic data known for four to six species provide the best estimates of dose for human toxicity, with a difference of calculated response to actual toxic response ranging from 1.5 to 3.4 times, i.e., the error in predicted dose will not be greater than 3- to 4-fold.

5.2.3 *Interspecies Scaling of the Concentration Curve*

In 1970 Dedrick *et al.* introduced the term "equivalent time". This concept is essentially the same as the ideas of "physiological time" (Brody, 1937) and "biological time" (Gunther and Guerra, 1955). The implications are that small animals are performing the same physiological functions as large animals only at a much faster rate. These functions include heartbeat, energy metabolism, chemical metabolism and chemical excretion. Thus, the fact that biological half-lives are longer for large animals than for small animals is to be expected. The use of this concept enabled Dedrick *et al.* to further advance the field of comparative pharmacokinetics.

Dedrick *et al.* (1970) described experiments using methotrexate in mice, rats, dogs, monkeys and humans. Single intravenous or intraperitoneal injections were given and plasma concentrations were determined. The dose range was 0.1–450 mg/kg. The body weights ranged from 0.022–70 kg. The plasma concentration range was from 0.0077 to 130 μg/ml.

Because the clearance varies considerably from one species to another, it is not surprising that the concentration–time curves for the five species studied were very different from one another in shape and magnitude. For a single species, the curves for different doses are similar in shape, but transposed more or less vertically from one another. Thus, each dose and species gives a separate curve which covers a range in concentration of more than four orders of magnitude (a factor of 17 000) over a period of nearly 7 h. These curves are shown in Figure 6.

Taking the ordinate as concentration, $C(t)$, and the abscissa as time, t, Dedrick *et al.* normalized the ordinate by dividing the observed plasma concentration by dose per unit body weight. They normalized the abscissa by dividing time for each species by $M^{1/4}$. When they plotted the same data from Figure 6 using these new units of concentration and time, they found that the data fit a single curve, as shown in Figure 7, given by

$$\frac{C(t)}{(D/M)} = \exp\left(\frac{-kt}{M^{1/4}}\right) \tag{73}$$

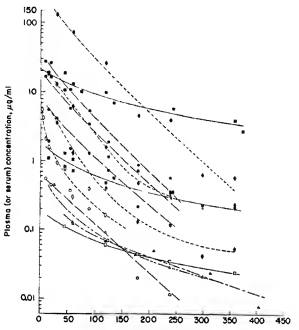

FIG. 6. Plasma (or serum) concentration of methotrexate in several species after i.v. or i.p. injection. (------) mouse; (————) rat; (– – – –) monkey; (— — —) dog; (———) human. (From Dedrick *et al.*, 1970; reproduced with permission of the publisher).

where k is the same for all species, M is the body mass, and D is the dose in milligrams.

The normalization of the time axis was accomplished by defining "equivalent time"; that is, Dedrick *et al.* noted that since the biological half-life for methotrexate for a mouse is about one-tenth the half-life for a human "methotrexate pharmacokinetics occur about an order of magnitude faster in mouse than man, and that an equivalent time might thus be defined." Thus, it could be said that 1 min to a mouse is equivalent to 10 min for a human, in terms of the amount of methotrexate eliminated. They proposed that the scaling factor could be approximated by the mean residence time or turnover time of the vascular system: blood volume divided by cardiac output. They then used data on these two parameters and found that the residence time was proportional to the body mass to the power 0.2. On the

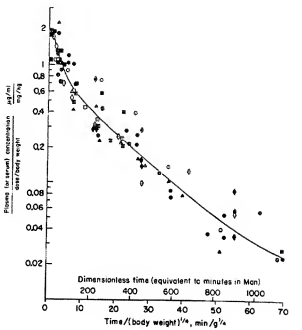

FIG. 7. Plasma or serum concentrations the data in Fig. 6 after normalizing the dose and converting to equivalent time. The symbols refer to different dose levels and routes of administration. (From Dedrick *et al.*, 1970, reproduced with permission of the publisher).

basis of other work, however, they decided to choose the exponent of one-fourth.

Dedrick *et al.* also pointed out that, since the data for all species fit this curve, the AUC for each species was invariant; that is,

$$\int (dt/M^{1/4})C/(D/M) = A, \tag{74}$$

where A is a constant for all doses and species. We can rearrange the above equation to obtain an expression for the usual area under the concentration–time curve (AUC) as follows:

$$\text{AUC} = \frac{AD}{M^{3/4}}. \tag{75}$$

This is similar to the result obtained by Mellet (1969), but consistent with the Kleiber (1932) and Brody and Proctor (1932) law rather than the surface law.

The difference from Mellet's result was explained by Dedrick *et al.* as a result of the fact that methotrexate is excreted mainly by the kidneys. Since inulin clearance varies as body mass to the power 0.77 (Adolph, 1949), it would be expected from eq. (65) that AUC would behave as in eq. (75).

This approach then, at least for methotrexate, brought consistency not only to the multiple-dose situation but also to the single-dose situation and simultaneously took plasma concentration, clearance and biological half-life into account. Of course, one question is why did it work? The reason can be seen from eqs (60), (69) and the Kleiber–Brody variant of eq. (72) which is

$$t_{1/2} = K M^{1/4}. \tag{76}$$

If, as is the case with methotrexate, the apparent volume of distribution, V, is proportional to M, then $C(0)$ is inversely proportional to M and dividing by D/M cancels all of the mass dependence of the coefficient of the exponential function. The exponential function itself involves $t/t_{1/2}$. From eq. (76) we see that this is proportional to $t/M^{1/4}$, therefore, plotting the new variable $T = t/M^{1/4}$ eliminates the mass dependence in the exponential function. Finally, the dose dependence in $C(0)$ was also cancelled when it was divided by D/M. Thus, the expression obtained is independent of mass and dose. The fact that all the data for five species with a range of mass over three orders of magnitude fits the same curve is certainly evidence of the validity of allometric equations for interspecies scaling. Whether a better fit would have been obtained using the surface area law, which means using eq. (72), was not tested; however, the fact that the surface area law would scale time with an exponent of 1/3, which is further from 0.2 than 1/4, would lead one to suspect that the fit would not be as good.

Recently, McGovren *et al.* (1988) have used the same technique to compare the pharmacokinetics of the antitumour agent acivicin, which is currently being evaluated in Phase II clinical trials. With one exception, plasma concentration–time data in six species over a 3000-fold body-mass range and a 120-fold dose range were fitted by the same normalized curve as that used by Dedrick *et al.* (1970).

It is interesting to note that the approach used by Dedrick *et al.* to estimate "equivalent time", that is the calculation of vascular residence time, was later discussed by Kleiber (1975). In this paper, he was considering the meaning of the metabolic rate divided by body mass, which, according to the Kleiber–Brody rule, would be proportional to mass to the power $-1/4$. Kleiber pointed out that in analogy to the concepts of turnover of contents of a pool (e.g. turnover rate of carbon atoms in an animal) the metabolic rate

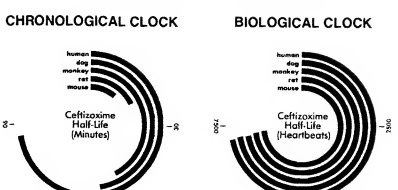

FIG. 8. Ceftizoxime terminal disposition half-lives referenced to chronological and heartbeat time (from Mordenti, 1985a; reproduced with permission of the publisher and the author).

per mass would be a turnover rate for energy. The inverse of that rate would be a residence time, and it would be proportional to the one-fourth power of the mass.

Mordenti has provided an imaginative graphical method for understanding physiological time (Fig. 8). She noted that while the disposition half-life of the antibiotic ceftizoxime has a wide species-to-species variation, it becomes invariant (as discussed in Sections 4.2.5) when divided by heartbeat time (Mordenti, 1985a, 1986b).

5.2.4 Excretion and Metabolism

Further evidence for the relationship between clearance and body mass was found by Klotz *et al.* (1976). They compared the pharmacokinetics of diazepam in humans, dogs, rabbits, guinea-pigs and rats. Diazepam and desmethyldiazepam, its major metabolite, were measured in blood and plasma after an i.v. bolus injection. The plasma concentrations were found to decay bi-exponentially in all species examined, and the two-compartment open model was used to analyse the data. They found the half-life to range from 1.1 h for rats to 32.9 h for humans with a monotonic behaviour as a function of mass. They did not attempt to find an allometric equation for half-life. However, they did find that the total plasma clearance was proportional to surface area using surface area values given by Spector

(1956). This result was particularly interesting in view of the fact that their data indicated that the clearance in humans was small compared to hepatic blood flow, whereas for dogs, rabbits and rats it was greater than blood flow, indicating a greater intrinsic ability to metabolize diazepam in these species relative to humans (indeed, they noted that extrahepatic elimination was occurring in the animals in order to have an extraction ratio greater than unity). In 1977, Weiss *et al.* reported on studies performed with an antiarrhythmic substance in which they made explicit use of allometry in the study of the dependence of clearance on body mass. They proposed that somewhat different allometric equations result, depending upon the elimination mechanisms involved. If the elimination is by metabolism, they noted that the hepatic clearance is given by

$$\mathrm{CL_H} = Q_\mathrm{H}(1 - C_o/C_i), \tag{77}$$

where $\mathrm{CL_H}$ is the hepatic clearance, Q_H is the hepatic blood flow, and C_i and C_o are the drug concentrations before and after liver passage, respectively. If the metabolism is complete after one passage, i.e. $C_o = 0$, then

$$\mathrm{CL_H} = Q_\mathrm{H} \tag{78}$$

and the metabolism rate is limited by hepatic blood flow. In that case, biological similarity considerations (see Section 4.4) would lead to the allometric equation

$$\mathrm{CL_H} = aM^{0.75}. \tag{79}$$

If metabolism is not blood flow limited, then the rate of elimination is determined in part by enzyme activity. Weiss *et al.* argued that the enzyme activity dependence on body weight involves an allometric equation with an exponent of 0.73 and concluded that in that case

$$\mathrm{CL_H} = aM^{0.73}. \tag{80}$$

They did not give a reference for this value; however, it would be very difficult to distinguish, experimentally, between 0.75 and 0.73.

If the drug is eliminated by the kidney and only through glomerular filtration, Weiss *et al.* argued that

$$\mathrm{CL_R} = \mathrm{GFR}, \tag{81}$$

where GFR is the glomerular filtration rate, which obeys an allometric equation with an exponent of 0.82.

Weiss *et al.* stressed that biological half-life is also an important parameter and noted that the equation

$$t_{1/2} = \frac{0.693V}{\mathrm{CL}}, \tag{82}$$

where V is the appropriate apparent volume of distribution, leads to an allometric equation for the biological half-life if V and CL obey allometric equations. They then proposed that if V is proportional to body mass, then for metabolism limited by hepatic blood flow

$$t_{1/2} = b_1 M^{0.25}, \tag{83}$$

and for renal elimination

$$t_{1/2} = b_2 M^{0.18}, \tag{84}$$

where b_1 and b_2 are constants. They then obtained eq. (61) for the relationship between maintenance dose and effective plasma concentration and noted that the ratio of the doses for two individuals or species necessary to maintain the same plasma level (assuming the same dosage interval) would be

$$\frac{D_1}{D_2} = \left(\frac{M_1}{M_2}\right)^{0.75}, \tag{85}$$

assuming that the clearance was governed by $\mathrm{CL_H} = Q_\mathrm{H}$. However, they also noted that since the concentration is proportional to the half-life divided by the distribution volume (see eq. (61)], for cases of repeated application the selection of a dosage interval that varied appropriately according to mass [e.g. eq. (83)] would lead to the equivalent doses being proportional to mass.

They applied these results to the pharmacokinetics of the particular drug they were studying (falirytmin) and found that the data could be fitted using the two compartment model and that the apparent distribution volume as well as the central compartment volume were proportional to mass. Applying eqs (73) and (82), they used the rabbit data to predict the half-life in a human and obtained 10.5 min, compared with a measured value of 13.6 min. Weiss *et al.* also noted that for some chemicals it may not be possible to use this approach for newborns because of a rapid change in distribution values in the first few weeks after birth. They concluded that it is generally possible (even when the elimination process is unknown) to scale clearance using an exponent of approximately 0.7 and half-life using an exponent of approximately 0.3.

In 1980 Boxenbaum collected data from the literature on the pharmacokinetics of antipyrine as well as liver weights and hepatic blood-flow rates in a range of species. These data were used to calculate hepatic clearance, where possible, by either dividing the dose by the area under the curve [eq. (65) or by using

$$\mathrm{CL} = \frac{0.693V}{t_{1/2}}, \tag{86}$$

which results from eqs (63) and (65) if bioavailability is total (i.v. or i.p. dose). The latter equation was used to calculate clearance only when half-life values were the only data available. In these cases, it was assumed that the apparent volume of distribution was equal to the total body water. Data were obtained for 11 species ranging in size from mice (30 g) to cattle (760 kg). Boxenbaum then showed that liver weight, hepatic blood flow and antipyrine intrinsic clearance obeyed allometric equations with good fits, except for the clearance data for humans who appeared to metabolize this drug much slower than animals. This inconsistency was later explained (Boxenbaum, 1984) by including brain weight as well as body weight in the allometric equation. This multivariate approach will be discussed in a later section.

As can be seen from the last two sections, the work of several investigators during the 1970s provided considerable support for the allometric scaling of pharmacokinetic data. These investigators showed that fundamental pharmacokinetic parameters such as clearance, half-life and distribution volumes for many drugs obey allometric equations. It is important to note, however, that the exponents involved can differ from one drug to another.

Since 1980, there have been several advances that have refined and extended the work by Mellet (1969), Dedrick *et al.* (1970), Weiss *et al.* (1977) and others. Many of these advances have been made by Boxenbaum, who, in a series of papers, has done much to clarify the concept of pharmacokinetic scaling. Boxenbaum (1982b) has emphasized that drug metabolism systems (and chemical metabolism in general) are the natural consequence of mechanisms developed initially to deal with naturally occurring xenobiotics. These compounds occur in many different forms, and animals have developed a large variety of strategies to detoxify the many chemicals encountered in their diets. As he noted (Boxenbaum, 1983), "we exist today in a biochemical and physiological state virtually identical to that in which we existed as a species some 300 thousand years ago" and in the study of interspecies variations in metabolism of chemicals "we are actually probing those evolutionary variations, adaptations, and nuances which developed so that species could adapt and survive."

5.2.5 Pharmacokinetic Time Scales

In a series of papers (Boxenbaum, 1982b, Boxenbaum and Ronfeld, 1983, Boxenbaum, 1986), Boxenbaum and his co-workers refined the concepts of physiological time first discussed by Brody in 1937 and the concept of pharmacokinetic scaling introduced by Dedrick *et al.* (1970). He re-emphasized the fact that the variation in biological half-lives with size and

the time scaling introduced by Dedrick *et al.* is a reflection of the faster tempo of living in small animals. When applied to pharmacokinetic events, he calls this new scaled time "pharmacokinetic time" and notes that it is not the same for all chemicals. A critical assumption made by Dedrick *et al.* (1970) was that the distribution volume was proportional to body mass. This is not the case for all chemicals (e.g. chlordiazepoxide has a smaller distribution volume per unit mass in a human than in a dog; Boxenbaum and Ronfeld, 1983).

Boxenbaum's approach can be easily demonstrated by using the one-compartment model. From eqs (60) and (69) we have for a single dose D at time $t = 0$

$$C(t) = (D/V) \exp(-kt), \tag{87}$$

where V is the volume of distribution. Furthermore, from eqs (67) and (86) we have

$$k = \frac{0.693}{t_{1/2}} = \frac{CL}{V}, \tag{88}$$

where CL is the clearance. Dedrick tacitly assumed that V is proportional to mass and that clearance is proportional to the three-fourths power of the mass. If, on the other hand (Boxenbaum and Ronfeld, 1983)

$$CL = aM^t \tag{89}$$

and

$$V = bM^y, \tag{90}$$

where M is the total body mass, we would then have

$$t_{1/2} = 0.693V/CL = (0.693\ b/a)M^{y-x}, \tag{91}$$

and unless $y - x = 1/4$, the scaling would differ from that used by Dedrick *et al.* (1970). Morover, if $y \neq 1$, the dose scaling would be different from that used by Dedrick. For the case where $y = 1$, Boxenbaum and Ronfeld (1983) use the term "elementary Dedrick plot" for the plot of $\ln [C(t)/(D/M)]$ versus (t/M^{1-x}) where the interspecies data for methotrexate (Dedrick *et al.* 1970) have been shown to be on the same curve. The time units which are given by t/M^{1-x} they called "kallynochrons".

For the case where y is not one, Boxenbaum and Ronfeld (1983) introduced the "complex Dedrick plot" whereby the data on plasma

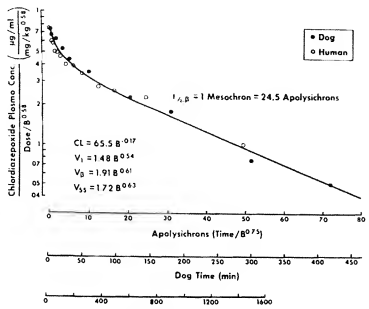

FIG. 9. Complex Dedrick plot of chlordiazepoxide plasma concentrations in dogs and humans. Time units are apolysichrons (from Boxenbaum and Ronfeld, 1983; reproduced with permission of the publisher and H. Boxenbaum).

concentration as a function of time is plotted as in $(C(t)/(D/M^y))$ versus t/M^{y-c}. This introduces a new unit of time (for $y \neq$ one) which they called the "apolysichron". Clearly, the apolysichron reduces to the kallynochron if $y =$ one. Figure 9 illustrates a concentration–time plot in terms of apolysichrons.

For the case of chronic dosing, we still have the result that the average concentration is given by [see eq. (65)]

$$C_{av} = \frac{(AUC)}{T} \tag{92}$$

or equivalently [eq. (66)]

$$C_{av} = \frac{D}{TCL}, \tag{93}$$

where AUC is the area under the curve for a single dose D, D is the dose

given chronically at intervals T, and CL is the clearance. All of these results assume that the entire dose reaches the systemic circulation; if the dose is given orally, then these results must be multiplied by the fraction of the dose reaching the systemic circulation. It can be seen that if the allometric equation for CL is determined, the plasma concentration for the chronic case depends only on x (at least for the one-compartment model). If, however, only the half-life is determined, the assumption that the allometric exponent volume (y) is equal to 1 may lead to incorrect scaling. One such example is given by chlordiazepoxide where (Boxenbaum and Ronfeld, 1983) found $y = 0.58$ and $x = -0.17$ for dogs and humans. This leads to

$$t_{1/2} = cM^{0.75}. \tag{94}$$

The use of eq. (88) and the assumption that $y = 1$ would lead to the clearance being proportional to the one-fourth power of the mass, whereas it is actually proportional to the -0.17 power of the mass. While this example is apparently a special case, it illustrates the possibility of being misled by assuming $y = 1$.

Boxenbaum (1982b) has further generalized the pharmacokinetic allometric approach by introducing brain weight into the scaling. It was noted earlier that the human antipyrine data did not scale correctly on the Dedrick plots. This is also true for other drugs as well (Boxenbaum, 1980; Yates and Kugler, 1986). Boxenbaum noted that humans also do not fit the allometric equation obeyed by most animals for maximum lifetime potential (MLP). MLP is the maximum documented longevity for a species. Sacher (1959) used multiple regression methods to develop the equation

$$\text{MLP} = 185.4(\text{BM})^{0.636}M^{-0.225}, \tag{95}$$

where MLP is in years and brain mass (BM) and body weight (M) are in kilograms. Table 19 gives the brain and body weights for various species.

In 1978, Boddington proposed that the metabolic rate times the longevity divided by the mass is a constant. He supported this claim with the allometric equations giving

$$\textit{metabolic rate} = \textit{constant} \times M^{0.75} \tag{96}$$

and

$$\textit{longevity} = \textit{constant} \times M^{0.25} \tag{97}$$

[as opposed to eq. (95), eq. (97) is not well fit by humans]. Boddington suggested that this result implies that an organism of given mass has a fixed total metabolism, and that the rate at which this metabolism potential is used up determines the longevity and time scale of the organism. Of course, this refers to the adult weight. Boxenbaum (1986) proposes that, in general,

TABLE 19

Mean body and brain weights for selected mammals*

Species	Body weight (kg)	Brain weight (kg)
Mouse	0.029	0.00042
Rat	0.23	0.0017
Guinea-pig	0.29	0.0036
Rabbit	2.93	0.012
Baboon	4.92	0.031
Monkey (rhesus)	5.82	0.077
Dog	14.2	0.075
Sheep	57.6	0.11
Pig	66.5	0.05
Human	70	1.53
Horse	398	0.57

* Adapted from Boxenbaum and Fertig (1984).

species have a predetermined fixed amount of "Phase 1 Hepatic Pharmacokinetic Stuff" per unit body weight; that is, the intrinsic hepatic clearance of the unbound drug times the MLP divided by the mass is a constant. This then leads to

$$\mathrm{CL_{int}} = \frac{aM}{\mathrm{MLP}}, \tag{98}$$

where a is a constant, and $\mathrm{CL_{int}}$ is the intrinsic clearance for the chemical of interest. The product of clearance and MLP is the total volume from which the drug would be cleared in a MLP assuming constant exposure. Yates and Kugler (1986) propose that this effect (the dependence on brain weight as well as body mass) is a reflection of the phenomenon of neoteny, the retardation of development or late maturation associated with some species including humans. It is interesting to note that Gould (1966), in his review of the effect of size and allometry on phylogeny and ontogeny, suggested that the simple one-variable allometric equation might, in some instances, have to be replaced by a multivariate equation. This suggestion has also been made by Yates and Kugler (1986).

In those cases (e.g. antipyrine; Boxenbaum and Fertig, 1984) where clearance is not proportional to a power of the body mass, but instead obeys the equation

$$\mathrm{CL} = aM^{z}\,(\mathrm{BM})^{z}, \tag{99}$$

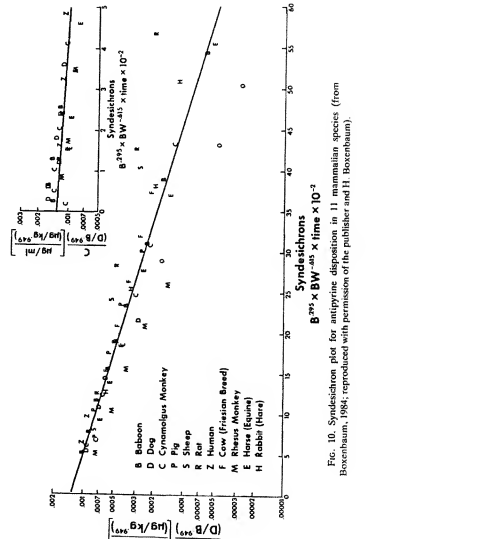

FIG. 10. Syndesichron plot for antipyrine disposition in 11 mammalian species (from Boxenbaum, 1984; reproduced with permission of the publisher and H. Boxenbaum).

where z is the allometric exponent for the brain mass, and the volume of distribution obeys

$$V = bM^{z}, \tag{100}$$

the complex Dedrick plots require the plotting of $C/(D/M^{z})$ versus $t/(M^{z-z'}(BM)^{-z'})$. Boxenbaum (1986) defines these time units as syndesichrons. Figure 10 illustrates a syndesichron plot for antipyrine in 11 mammalian species.

These concepts have been extended to the two compartment model (Boxenbaum, 1986) where the volume of distribution and half-life are referenced to the terminal phase.

5.3 APPLICATIONS TO INTERSPECIES SCALING OF PHARMACOLOGICAL AND TOXICOLOGICAL DATA: THE ALLOMETRIC AND PB-PK MODELS

Mordenti (1986b) has recently compared two approaches to interspecies pharmacokinetic scaling (there is also an excellent discussion of the relative merits of these techniques in Boxenbaum and D'Souza, 1990). These are the allometric approach and the physiological approach. The allometric approach involves the development of allometric equations for individual fundamental parameters such as distribution volumes and clearance. It does not require a detailed knowledge of organ distribution, enzymatic activities and blood-to-plasma concentration ratios. It does assume, however, that the pharmacokinetics of the chemical are first-order, the percentage of protein binding is similar in the species involved and linear, the elimination processes are renal-, biliary-, or flow-limited and that sufficient data are available for linear regressions (Mordenti, 1986b).

The physiological approach involves detailed mass-balance calculations for a flow model describing the absorption, distribution, metabolism and excretion of the chemical. This flow model connects all the important compartments (e.g. brain, heart, liver, kidney, muscle, fat, etc.) to the circulatory system. The resulting differential equations are solved. The solutions are applied to a test species to determine the adequacy of the model and fit any undetermined parameters. The predictions for humans are then obtained by replacing the values for flow rates, compartment volumes, etc., for the test species by those for humans. These values might be obtained from the literature, from *in vitro* experiments, or via allometric scaling.

5.3.1 The Allometric Model

The allometric approach is the same as that used by Dedrick (1970), Boxenbaum (1986) and others. Mordenti (1985b) has illustrated the use of this technique in the prediction of ceftizoxime concentration profiles in humans. This was done by taking reported values for the parameters in the two-compartment model.

The rate constants (a, b) and the coefficients (A, B) in the bi-exponential equation for the serum concentration following a single bolus dose,

$$C(t) = A \exp(-at) + B \exp(-bt), \tag{101}$$

were obtained for ceftizoxime in mice, rats, monkeys, and dogs. Allometric equations for A, B, a and b were then generated by using linear least-squares analysis on the log-transformed data, and values for each parameter in a 70 kg human were calculated. Serum concentration curves were constructed for a 1 g i.v. bolus and a 4 g infusion by correcting for the dose used in the preclinical and clinical studies as follows:

$$C(t) = \left(\frac{D_h}{D_a}\right)[A \exp(-at) + B \exp(-bt)], \tag{102}$$

where D_h and D_a are the dose in human and the dose in animal, respectively. Mordenti (1986a,b) then compared the predicted result to the results obtained by both a 1 g i.v. bolus and a 4 g infusion of ceftizoxime and found very good agreement over periods up to 8 h for the bolus and 12 h for the infusion injection. The half-life for humans in the terminal phase is 2 h.

Mordenti also investigated two other methods for calculation. One involved obtaining allometric equations for k_{12}, k_{21} and k_{10}, the rate terms in the two-compartment model for the central and peripheral compartments. The other method involved obtaining allometric equations for the clearance and distribution volumes. She obtained consistent results by all three approaches. The approach involving the direct use of A, B, a and b is the easiest, according to Mordenti, and is well-suited to multi-exponential models. In this case, because the protein binding is low, she used total serum concentration. Where the protein binding is high or there is great interspecies variation, she suggested the use of unbound concentrations in the serum. She also pointed out that renally excreted compounds are easier to scale. For a compound which undergoes metabolism, she suggested that in order to proceed the investigator must decide what is the most important factor in the system; that is, is the disappearance of the parent compound or the appearance of the metabolite or the elimination route the most important factor?

If toxic effects correlate with average steady-state concentrations, then a slightly modified form of eq. (93) is obtained

$$C_{av} = \frac{FD}{TCL}, \tag{103}$$

where F = fraction of dose absorbed (i.e. bioavailability) and C_{ss}, D, T, CL are in units of milligrams per millilitre, milligrams, hours and millilitres per hour, respectively (or equivalent units). This equation can be used (Mordenti and Chappell, 1990) to scale from animals to humans as follows:

$$C_h = \frac{F_h D_h}{T_h \mathrm{CL}_h} \tag{104}$$

and

$$C_a = \frac{F_a D_a}{T_a \mathrm{CL}_a}, \tag{105}$$

where eqs (104) and (105) are the equations for the human (denoted by subscript h) and animal (subscript a), respectively. Assuming equal average concentrations lead to equal effects, we set $C_a = C_h$ to obtain

$$D_h = D_a\left(\frac{F_a}{F_h}\right)\left(\frac{T_h}{T_a}\right)\left(\frac{\mathrm{CL}_h}{\mathrm{CL}_a}\right). \tag{106}$$

If T, the dose interval, is the same for animals and humans, and F, the bioavailability, is the same, then

$$D_h = D_a\left(\frac{\mathrm{CL}_h}{\mathrm{CL}_a}\right). \tag{107}$$

Assuming that clearance scales with an allometric exponent of 0.7 (Chappell, 1989), the relation between doses becomes

$$D_h = D_a\left(\frac{M_h}{M_a}\right)^{0.7}. \tag{108}$$

As noted by Mordenti and Chappell (1990), toxicity sometimes occurs only when a threshold concentration is exceeded. In such a case it is better to scale peak concentrations, and the equivalent dose for a single dose is given by

$$C_p = \frac{FD}{V}, \tag{109}$$

where C_p is the peak concentration (milligrams per millilitre), F is the bioavailability (as a fraction), D is the dose (in milligrams, for example) and V is the volume of distribution (in millilitres). For equivalent peak concentrations the relationship is given by

$$\frac{F_h D_h}{V_h} = \frac{F_a D_a}{V_a}. \tag{110}$$

Assuming equivalent bioavailabilities and that the volume of distribution scales as $M^{1.0}$, the equivalent human dose is given by

$$D_h = \frac{D_a M_h}{M_a}. \tag{111}$$

Thus, assuming that the allometric equation for volume scales as $M^{1.0}$, the situation for peak concentration equivalency scales neither as $M^{0.67}$ or as $M^{0.75}$, but instead each species requires the same dose in milligrams or micrograms per kilogram for equivalency. While volumes of distribution often scale as $M^{1.0}$, this is not always true (Boxenbaum and Ronfeld, 1983).

Mordenti (1986a) has proposed a method for situations where it is important to have equivalent AUC and peak concentration. Equivalent exposure or pharmacokinetic equivalency was defined as having the same peak serum concentration and the same area under the serum concentration curve (AUC) in a finite dosing interval. She used the drug ceftizoxime as an example to demonstrate how to determine the dose and dose interval which would give equivalent exposures to different species. Mordenti (1986a) noted that if the serum concentration is described by the two-compartment model [eq. (101)] then the coefficients (A and B) are proportional to the dose. The peak concentration, $C(0)$, and the 24 AUC for a 1 g bolus dose given every 8 h to a 70 kg human were used as standards. For this dosage regimen, $C(0) = 0.141$ mg/ml and AUC = 0.479 mg h/ml. If a dose D_0 (in mg/kg) was used in the initial studies to determine A and B in eq. (101), then the dose (D) which will produce $C(0) = 0.141$ mg/ml is

$$D = \frac{D_0 \times 0.141\ (mg/ml)}{(A + B)}. \tag{112}$$

The AUC is then obtained for the test animal for the new dose, D (for a single dose). It is assumed that the pharmacokinetics are still first-order at this new dose. Mordenti then proposes that to obtain the same AUC for the animal as the value 0.479 mg h/ml for humans, N doses of size D should be given in 24 h where

$$N = \frac{\mathrm{AUC_h}(24\,\mathrm{h})}{\mathrm{AUC_a}(1\,\mathrm{dose})}, \tag{113}$$

where $\mathrm{AUC_H}$ (24 h) and $\mathrm{AUC_a}$ (one dose) are the areas under the curve for the human for a 24 h interval and for the animal for one dose.

Table 20 gives the values Mordenti obtained for the number of doses and the dose size for this case. The allometric equations obtained for the dose and the dosage interval are given by:

$$\text{dose (mg/kg)} = 35M^{-0.20} \tag{114}$$

TABLE 20

Pharmacokinetic equivalent dosing regimens for ceftizoxime in animals and humans[a]

Parameters	Species					Allometric equation
	Mouse	Rat	Monkey	Dog	Human	
Weight (kg)	0.023	0.18	7.5	12	70	—
Dose (mg/kg)	88.1	37.5	24.3	23.6	14.3	$35M^{-0.20}$
No. of doses/24 h	20	15	7	4	3	$8.9M^{-0.24}$
Dosage interval (h)	1.2	1.6	3.4	6	8	$2.7M^{0.24}$

[a] From Mordenti (1986a).

$$dosage\ interval\ (h) = 2.7M^{0.24}. \tag{115}$$

It can be seen that the dose in milligrams is proportional to mass to the power 0.8 rather than 0.67 (surface law) or the 0.75 power (Brody–Kleiber law). We also see that the dosage interval is proportional to the one-fourth power of the mass. Thus, as noted previously, small animals require larger doses (in milligrams per kilogram) of drugs, administered more frequently, than larger animals or humans to give the same peak concentration and the same AUC. Clearly, the need for more frequent administrations in smaller animals is due to the much faster clearance in smaller animals. Mordenti (1986a) points out that if the allometric expression for ceftizoxime half-life is divided by that for heartbeat time, an interspecies invariant is obtained such that 50% of the dose is eliminated in about 7300 heartbeats regardless of the species (Fig. 8). Mordenti suggests that for some toxicological or pharmacological studies small mammals may be impractical because of the frequency of administration required.

Mordenti and Chappell (1990) have noted that in the case where pharmacokinetic studies have not yet been performed, the approximation $CL = M^{0.7}$ can be used to solve for AUC giving

$$N_h = N_a\left(\frac{F_a}{F_h}\right)\left(\frac{D_a}{D_h}\right)\left(\frac{M_h}{M_a}\right)^{0.7}, \tag{116}$$

where N_a and N_h are the number of doses per day for the animal and human, respectively, to obtain equivalency. It is assumed that the pharmacokinetics of the drug remain first-order at all dose levels for these equations to be valid.

Mordenti (1986a) notes that pharmacokinetic equivalency may not always be the same as toxicological equivalency. Assuming that it is, many short-term toxicological studies where different species received doses at the same intervals, or intervals that are not properly related to pharmacokinetic time, may give results which cannot be reliably extrapolated to humans. This situation could have important consequences for the 1 day and 10 day health advisories that the US Environmental Protection Agency develops on the basis of animal studies.

Mordenti again cautions that these approaches are much more straightforward for renally excreted compounds than for compounds which are metabolized. This is especially true for compounds where the metabolites are responsible for the toxic effects. In such cases it is conceivable that smaller species will be affected by smaller doses (milligrams per kilogram) than larger species. Indeed, Conolly and Andersen (1990) have proposed that in many instances the toxic effects of reactive metabolites will scale as the inverse of the surface area.

Mordenti (1986b) proposes a five step approach to use the allometric method to predict pharmacokinetic profiles for humans from animal data. These five steps are:

(1) Determine the individual pharmacokinetic parameters for the drug in young adult animals of four or more species.
(2) Perform linear regression analysis on the logs of these parameters versus log total body mass (and, if necessary, brain weight or other parameters).
(3) Solve each equation for humans by setting $M = 70$ kg.
(4) Use these parameters to predict drug disposition in humans.
(5) Check the prediction by administering the drug to young adult humans or comparing it to data in the literature.

Similar approaches have been successful in predicting pharmacokinetic parameters in humans. We have already mentioned that the work of Swabb and Bonner (1983) and McGovren *et al.* (1988). Sawada *et al.* (1985) predicted disposition half-life, metabolic clearance, distribution volume and unbound fraction in the plasma of nine weakly acid and six weakly basic drugs. These predictions, based on data from rats, were successful for most of the drugs considered.

5.3.2 *The Physiological Model*

The physiological approach has been used with some success to describe situations where non-linear dynamics occur or where the biological response is not a function of the total or unbound concentration in the plasma (Anderson *et al.*, 1987). This approach is now frequently described as

physiologically-based pharmacokinetics (PB-PK). While requiring far more data and effort than the allometric approach, the proponents of the PB-PK method believe it holds great promise for interspecies extrapolation. As noted in a recent paper by Boxenbaum and D'Souza (1990), the PB-PK approach is a reductionist paradigm, "reducing" organisms to their constituent parts. Allometric scaling, on the other hand, "is predominantly empiric" (Boxenbaum and D'Souza, 1990).

There are several review articles on the PB-PK method (NAS, 1987; Chen and Gross, 1979; Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983; Rowland, 1985). The PB-PK models are mass-balance models in which it is generally assumed that organs and tissues with similar behaviour can be lumped together into compartments and connected by the fluid motion between and through the compartments; figure 11 illustrates one such model. Each compartment can be considered to have three sub-compartments consisting of a vascular section, an interstitial space, and a cellular space (Gerlowski and Jain, 1983). Additional assumptions are then

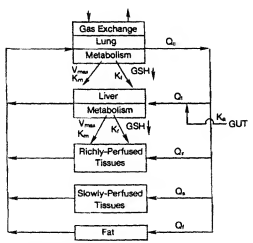

FIG. 11. A physiologically-based pharmacokinetic model for ethylene dichloride (EDC). The biodistribution of EDC was modelled by dividing body tissues into three compartments based on their blood flow (Q_r, Q_s and Q_f) and relative ability to accumulate EDC. The liver and lung are considered the only metabolizing organs. V_{max} and K_m are the rate constants for the oxidation pathway, and K_i is the rate constant for the GST pathway. The arrows denote the direction of blood flow. (From D'Souza *et al.*, 1987; reproduced with permission of the publisher.)

made about the necessity to treat these subcompartments as separate and about the transport mechanisms, binding, excretion and metabolism of the chemical being considered. Himmelstein and Lutz (1979) noted that four kinds of information are required: anatomical (e.g. organ volumes), physiological (e.g. blood flow rates and enzyme reactions), thermodynamic (e.g. binding isotherms) and transport (e.g. membrane permeabilities). Differential equations describing the mass balance around each compartment are written. These contain terms for inflow, outflow, production, and destruction of the chemical and its metabolites. The equations are then solved using appropriate algorithms. Values for the various constants are obtained from the literature or determined to fit the animal models. When the various coefficients in the equations are replaced by human values, a prediction is obtained for humans.

Clearly, the PB-PK method is far more elaborate, time-consuming, data-intensive and costly than the other approaches described in this chapter. However, there are many examples where the application of this method seems to have been successful. It is interesting to note that allometric scaling is often used to substitute for various physiological parameters such as cardiac output and ventilation rate (Andersen *et al.*, 1987).

One general situation where this approach has application is where saturation can occur. This might occur because of the finite capacity of protein binding sites or because of saturation of metabolizing enzymes. Other similar saturation conditions can frequently be modelled by replacing the first-order constants, K, that appear in rate equations in terms such as

$$\frac{dC}{dt} = -kC \tag{117}$$

by the Michaelis–Menten model (Gerlowski and Jain, 1983), where

$$\frac{dC}{dt} = -\frac{V_{max}C}{K_m + C}. \tag{118}$$

The constants V_{max} and K_m are the Michaelis–Menten constants. V_{max} is the maximum rate of the reaction. At low concentrations eq. (118) reduces to eq. (117) with

$$k = V_{max}/K_m, \tag{119}$$

and at high concentrations saturation occurs and

$$\frac{dC}{dt} = -V_{max}. \tag{120}$$

The Michaelis–Menten term introduces a non-linearity into the differential equations and therefore violates the assumptions that lead to the plasma concentration being proportional to dose.

Another situation is where the biological response does not depend upon the plasma concentration but instead depends on the concentration in a particular tissue (e.g. Cd in the kidney). In that case, the differential equation for the concentration in a typical tissue compartment is

$$V\left(\frac{dC}{dt}\right) = QC_p - Q(C/R) - K(C/R) - \frac{V_{max}C}{K_m + C}, \quad (121)$$

where C and C_p are the concentrations of the chemical in the compartment and plasma, respectively; V and Q are the volume and blood flow rate, respectively; K is the first-order rate constant and V_{max} and K_m are the Michaelis–Menten constants; R is the ratio of C to the venous plasma concentration. Clearly, eq. (121) leads to a nonlinear dependence of C upon C_p, except for small values of C.

In the case of active metabolites, the PB-PK approach allows for the consideration of these compounds. Whereas the factors considered for the allometric model for the original compound are not, in many cases, relevant, it is possible that allometric scaling could be applied to the metabolites if sufficient data is available. This is potentially important because it could be argued that the faster metabolism often associated with small animals will make them more vulnerable (on a mg/kg basis) than humans, because of the relatively higher production of metabolites.

There is no simple way to decide exactly how many body regions or compartments are needed. One must base the initial choices upon whatever is known about the physiochemical properties (binding, lipid solubility, ionization) and pharmacological properties (mechanisms of transport, site(s) of action) of the drug (Himmelstein and Lutz, 1979).

Once the body regions have been selected, the mathematical pharmacokinetic model is obtained by writing mass-balance equations for the sum of the processes occurring in each compartment. Important parameters in the physiological model include the blood flow to eliminating organs; tissue and fluid volumes; blood-to-plasma and tissue-to-plasma drug concentration ratios; drug protein binding; and enzyme kinetics.

The amount of drug in the blood of a physiological model is as follows (Bischoff, 1986):

accumulation of unbound drug + accumulation of bound drug
= flow in from organs
− flow out from organs + new i.v. doses. (122)

This relationship is written mathematically (Bischoff, 1986) as follows:

$$w_B V_B(dC_B/dt) + \rho_B V_B(dX_B/dt) = \Sigma\{Q_i[w_{Bi}C_{Bi} + \rho_{Bi}X_{Bi}] - (Q_B)[w_B C_B + \rho_B X_B]\} + (\text{new i.v. doses}), \quad (123)$$

where C is the free (unbound) concentration; X, bound concentrations; V, volume; Q, flows; w, fraction water; ρ, protein concentration; and the subscripts B and i are blood pool and the i-th tissue region, respectively.

If membrane transport is by simple diffusion (i.e. the free concentration is the driving force), the amount of drug in equilibrium blood in a tissue includes a diffusion-mediated membrane transport term, $k_i(C_{Ti} - C_{Bi})$, where k is membrane permeability and T is tissue. If facilitated active transport is operational, the membrane transport term may have a different form. The relationship for simple diffusion is written mathematically as follows:

$$w_{Bi}V_{Bi}(dC_{Bi}/dt) + \rho_{Bi}V_{Bi}(dX_{Bi})/dt = Q_i(w_B C_B + \rho_B X_B - w_{Bi}C_{Bi} - \rho_{Bi}X_{Bi}) + k_i(C_{Ti} - C_{Bi}). \quad (124)$$

The amount of drug in a tissue includes a term for reaction, secretion, reabsorption, etc., $r_i(C_{Ti}, X_{Ti})$, where r is a rate constant and T is tissue. This term can have a positive or negative sign, depending on the net effect of the process. This relationship is written mathematically (Bischoff, 1986) as follows:

$$w_{Ti}V_{Ti}(dC_{Ti}/dt) + \rho_{Ti}V_{Ti}(dX_{Ti}/dt) = k_i(C_{Bi} - C_{Ti}) + r_i(C_{Ti}, X_{Ti}). \quad (125)$$

It should be noted that each compartment can be divided into three subcompartments consisting of a vascular space, an interstitial space and an intracellular space, and equations have been presented to describe the mass balance in these compartments (Bischoff, 1987; Tsuji *et al.*, 1983).

The formal procedure for solving flow-limited models is to let k approach infinity, making C_{Ti} approximately equal to C_{Bi}, in which case both of these concentrations can be replaced by C_i (the free concentrations; Bischoff, 1986). This assumption is based on the simple form of eq. (124) with diffusion-mediated membrane transport, and permits combining eqs (124) and (125) into the following equation:

$$(w_{Bi}V_{Bi} + w_{Ti}V_{Ti})(dC_i/dt) + \rho_{Bi}V_{Bi}(dX_{Bi}/dt) + \rho_{Ti}V_{Ti}(dX_{Ti}/dt) = Q_i(w_B C_B + \rho_B X_B - w_{Bi}C_i - \rho_{Bi}X_{Bi}) + r_i(C_i, X_{Ti}). \quad (126)$$

Sets of tissue equations (126) are solved together with eq. (123).

When detailed binding information is available, the relationship between

bound and free drug can often be represented by a Langmuir isotherm (Bischoff and Dedrick, 1968). Detailed binding information is not always available, and a further useful simplification is that of linear binding, either exactly or approximately. Then the basic mass-balance equations are further condensed and written in terms of total drug concentrations, C^*, which is equal to $C_{free} + C_{bound}$ as follows (Bischoff, 1986):

$$V_B(dC_B^*/dt) = \Sigma[Q_i C_i^*/R_i] - (\Sigma Q_i C^*)B + (\text{new i.v. doses}), \quad (127)$$

where R_i is the tissue-to-blood distribution ratio constant. C_i obeys a similar equation with the free concentration as a driving force, i.e.

$$(V_{Bi} + V_{Ti})(dC_i^*/dt) = Q_i(C_B - C_i^*/R_i) + r_i(C_i^*). \quad (128)$$

Once the pharmacokinetics of the drug are defined in one animal species, predictions for humans are obtained by replacing the physiological, anatomical, biochemical and thermodynamic parameters used for the experimental animal species with the values for the corresponding parameters from humans. These values can be obtained from the literature, determined from *in vitro* experiments or estimated via interspecies allometric extrapolations. Some data, such as tissue-to-plasma drug partition coefficients, are not readily available for humans, and one sometimes assumes that the values in humans are the same as the values in the experimental animal species, which may not always be true (Tsuji *et al.*, 1985). Membrane permeabilities are sometimes assumed to be less important than the amount of drug presented to a region—the so-called "flow-limited" approximation (Bischoff, 1986); however, models utilizing actual values of membrane permeabilities are sometimes required (Bischoff, 1975; Lutz *et al.*, 1980). Bischoff (1987), Tsuji, *et al.* (1983) and Bischoff (1975) presented detailed mass-balance equations for blood and tissues.

As an example of the value of the method, a physiological model for penicillin V was developed in rats (Tsuji *et al.*, 1979). The model had nine compartments; blood, kidney, liver, heart, spleen, muscle, poorly perfused tissues, gut wall and gut contents. The model accounted for renal elimination, hepatic metabolism, biliary secretion and enterohepatic circulation. The assumptions in the model were as follow:

(1) Each tissue acts as a well-stirred compartment.
(2) The antibiotic distribution is limited by the blood flow.
(3) The tissue-to-blood concentration ratio of penicillin V is independent of the antibiotic concentration.

Penicillin V was administered as a bolus dose or as a constant infusion to steady state. Predicted penicillin V concentrations and amounts in blood, urine, gut contents and tissues of the rat agreed well with the experimental data. The steady-state data were particularly useful in establishing the clearance values for the various elimination pathways.

A PB-PK model for cefazolin was developed in rats (Tsuji *et al.*, 1983). The model had nine compartments in which the lungs, heart, muscle, skin, gut and carcass were modelled as cell membranc limited; the liver and kidney were modelled as flow-limited; and the bone compartment was divided into marrow (flow-limited) and cortex (membrane-limited). Important features of the model were non-linear protein binding and non-linear biliary excretion. Probenecid did not effect the urinary secretion of cefazolin; therefore, it was assumed that secretion was not a significant component of renal elimination. Erythrocyte uptake, serum protein binding, and tissue-to-plasma partition coefficients were determined *in vivo* and included in the model. In independent experimcnts, the model accurately predicted the disposition of cefazolin in rats following intravenous bolus administration.

The cefazolin physiological model described above was modified for rabbits by incorporating species-specific values for non-linear albumin binding, renal clearance, tissue volumes and tissue plasma flows (Tsuji *et al.*, 1985). The model successfully predicted the disposition of cefazolin in normal and renally ligated rabbits (Fig. 12). The authors scaled the cefazolin physiological model to humans and satisfactorily predicted bone and plasma

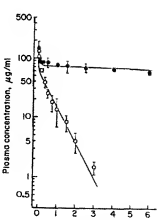

FIG. 12. Model-predicted (lines) versus observed (circles) plasma cefazolin concentrations in rabbits after a 10 mg/kg i.v. bolus dose to normal (○) and renally ligated (●) animals (from Tsuji *et al.*, 1985; reproduced with permission of the publisher).

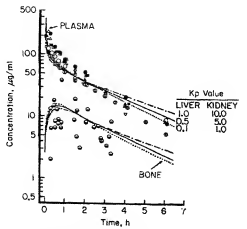

FIG. 13. Model-predicted (lines) versus observed (circles) cefazolin concentrations in bone (lower set of curves) and plasma (upper set of curves) after a 1 g i.v. bolus dose given to human subjects who were undergoing total hip replacement surgery. Predictions obtained by varying the tissue-to-plasma partition coefficient (K_p) demonstrates the sensitivity of this parameter in the model. (From Tsuji, *et al.*, 1985; reproduced with permission of the publisher.)

concentrations (Fig. 13). This work underscores the value of physiological models in evaluating antibiotic doses and dosing regimens for infections that occur outside the intravascular space. Conventional one- and two-compartment models employing plasma curve-fitting techniques cannot yield comparable information about the extravascular distribution of antibiotics.

An outstanding feature of the PB-PK model is the ability to predict the influence of disease, concomitant medications, age, pregnancy or dosage form on drug disposition. As illustrated in Figure 12, the physiological model for cefazolin (Tsuji *et al.*, 1985) was useful in predicting the effects of renal impairment on drug elimination. In another example, an 11-compartment PB-PK model was developed in rats to study the drug interaction of tolbutamide, a sulphonylurea derivative, with three sulphonamides: sulphaphenazole, sulphadimethoxine, and sulphamethoxazole (Sugita, 1982). The concentration of tolbutamide in several tissues and plasma was predicted using *in vitro* estimates of the plasma-binding and metabolic parameters and *in vivo* determinations of plasma-to-blood concentration ratios and tissue-to-plasma unbound concentration ratios. The predicted concentration curves of tolbutamide in plasma and in each tissue showed good agreement with the observed values except for the

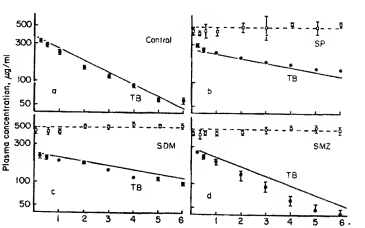

FIG. 14. Predicted and observed plasma concentrations of tolbutamide (TB) after intravenous administration of 80 mg/kg in the presence or absence of sulphonamides in rats. Each point and vertical bar represent the mean ± SE of three rats. Clockwise from upper left panel: control (tolbutamide alone); tolbutamide with sulphaphenazole (SP); tolbutamide with sulphamethoxazole (SMZ); and tolbutamide with sulphadimethoxine (SDM). ●, plasma concentration of tolbutamide; □, plasma concentration of the sulphonamide; ——, predicted concentration of tolbutamide; – – –, mean concentration of each sulphonamide for a 6 h constant infusion. (From Sugita *et al.*, 1982; reproduced with permission of the publisher.)

brain, for which the predicted concentrations were lower than the observed values in the early time period (Figs 14 and 15).

In the area of toxicity extrapolation, there have been several applications of the PB-PK model in recent years. Two recent papers provide examples of the use of this approach not only in extrapolation from animals to humans, but also in extrapolating from high to low doses in the laboratory animal.

In a recent paper, Anderson *et al.* (1987) describe the application of the PB-PK model to methylene chloride (dichloromethane, DCM). They noted that carcinogenicity studies had given rise to equivocal results. They proposed a model that was based on another model developed by Ramsey and Andersen (1984). This model has seven compartments: gas exchange, lung metabolism, richly perfused tissue (e.g. kidney and brain), slowly perfused tissue (e.g. muscle), fat, liver, and gastrointestinal tract. On the basis of data about the metabolism of DCM, they assumed two metabolic pathways. One pathway was the formation of formyl chloride in the cytochrome P-450 (mixed function oxidase, MFO) pathway. The other was

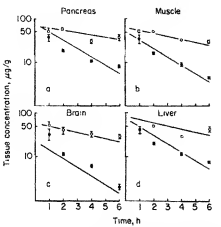

FIG. 15. Predicted and observed tissue concentrations of tolbutamide after intravenous administration of 80 mg/kg in the presence or absence of sulphaphenazole (SP) in rats. Clockwise from upper left: pancreas, muscle, liver, brain. Each point and vertical bar represent the Mean ± SE of three rats. ●, tissue concentration of tolbutamide without sulphaphenazole; ○, tissue concentration of tolbutamide with sulphaphenazole; – – –, predicted concentration of tolbutamide. (From Sugita *et al.*, 1982; reproduced with permission of the publisher.)

the formation of chloromethyl glutathione (GSH) in the cytosolic GSH-*S*-transferase (GST) pathway. They treated the GST pathway with first-order kinetics and the MFO pathway as saturable using the Michaelis–Menten model. Differential equations were written for the model and solved to obtain results for the AUC in the lungs and liver (the site of the tumours) for the DCM in blood, the AUC for DCM in the liver and lung, and the concentrations of the metabolites in the lung and liver.

The results indicate that the doses at which tumours were caused in the laboratory animals were such that the MFO pathway had been saturated, leading to a greater production of the GST metabolite which was apparently the carcinogenic compound. As a result, the linear extrapolation to low doses, where the MFO pathway is nearly first-order, apparently severely over-estimated the response because of the non-linearity. The inhalation and oral dose experiments gave rise to very different results because the oral doses did not saturate the MFO pathway.

Anderson *et al.* (1987) used *in vitro* data to obtain partition coefficients (e.g. blood–air) and obtained physiological constants such as tissue volumes and blood flows from the literature and laboratory studies. Biochemical constants such as K_m and V_{max} were obtained from the literature and

laboratory studies. They validated the model by comparing predictions of blood concentrations with results from experiments on animals and humans. The model gave very good fits to blood concentration data and to data on the appearance and elimination of DCM metabolites.

Another recent paper by D'Souza *et al.* (1987) describes the application of the PB-PK model to ethylene dichloride (EDC), which is also metabolized by two competing pathways. One is a saturable pathway involving cytochrome P-450 oxidation and the other involves direct conjugation by GST.

Laboratory results showed that the GST pathway is first-order until high EDC doses cause a depletion of GSH, decreasing the GSH available to react with EDC. D'Souza *et al.* (1987) used a model containing second-order terms to simulate the GSH depletion (see Fig. 11). When these considerations were incorporated into a PB-PK model similar to that just described for DCM, results were obtained that were consistent with carcinogenicity data. In this case, the glutathione conjugate was used in the liver as the dose surrogate. D'Souza *et al.* found the same non-linearity as Anderson *et al.* (1987) had for DCM. But at even higher doses, they found that the GST pathway is also overwhelmed because of depleted GSH concentrations. As a result, at very high doses, the amount of glutathione conjugate is proportionately lower than the administered dose (Fig. 16).

Thus, the PB-PK models appear to have several advantages. They have the potential of describing situations where non-linearities occur. Although it is possible that the allometric approach could also describe non-linear

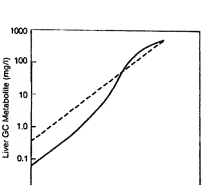

FIG. 16. The relationship between ethylene dichloride oral dose and the dose surrogate in the mouse (——) and a direct extrapolation from the 150 mg/kg dose assuming a 1 : 1 relationship (– – –). (From D'Souza *et al.*, 1987; reproduced with permission of the publisher.)

behaviour, it has not been tried. The PB-PK models have also been used to model more appropriate surrogates than plasma concentration, although there seems to be no reason why the allometric models cannot do the same. The PB-PK models also seem to have some success in extrapolating from high to low dose, a source of great uncertainties in many models. On the other hand, these are very sophisticated models and not many people have been trained in their development, use and interpretation; furthermore, the data required can quickly run up the expense of performing risk assessments. Nevertheless, there are probably situations where these models are appropriate. It would be very useful indeed to develop a set of criteria for deciding when a PB-PK model is needed and when simpler models will suffice.

6 Discussion and Conclusions

In recent years, the field of pharmacology has focussed considerable attention on the problem of interspecies scaling. This attention developed very naturally with the rapid expansion in drug synthesis and the need to determine the therapeutic and toxicological effects of new drugs. For a considerable period, there was a search to find a test species that metabolizes drugs or even classes of drugs in the same way as in humans, but this search has been futile and no single animal model of drug pharmacokinetics in human has been found. Indeed, it has become clear that small animals rarely, if ever, metabolize drugs at the same rate as humans. This difference in metabolic rates is apparently a reflection of the phenomenon of physiological or pharmacokinetic time; that is, the equivalent time in terms of life processes in a rat is much shorter than that in a human. This phenomenon is reflected by shorter lifespans and shorter biological half-lives of drugs in smaller animals when compared to larger animals. It is possible that the concept of physiological time is the fundamental underlying concept in interspecies extrapolation, rather than surface area, elastic similarity or the various other similarity concepts discussed in earlier sections. On the other hand, the history of extrapolation has demonstrated that an over-reliance on an attractive conceptual framework can lead to tunnel vision.

The faster clearance of chemicals in smaller animals has profound consequences on the interspecies scaling of the pharmacokinetics of chemicals. This phenomenon leads to the need for higher (in milligrams per kilogram body weight) and more frequent doses to achieve similar plasma concentrations in small animals, compared with larger animals. In the mid

1960s and early 1970s, investigators began to appreciate the fact that clearance per unit mass was much larger in small animals than in large animals. At about that time, several investigators (Fujita *et al.*, 1966; Mellet, 1969; Dedrick *et al.*, 1970) found evidence suggesting that the biological half-life and clearance for many drugs were proportional to some power of the body mass. That is, these fundamental pharmacokinetic parameters obey allometric equations. The use of the relationships was shown to lead to consistency between species in the pharmacokinetics of several drugs.

Compared to the previous work by Crawford *et al.* (1950), Talbot *et al.* (1953) and Freireich *et al.* (1966), the prediction of plasma concentrations by means of allometric equations for fundamental pharmacokinetic parameters, such as half-life, clearance and distribution volumes, was a considerable advance in the understanding of the fundamental mechanisms involved; however, as Mordenti (1986b) noted, the allometric pharmacokinetic approach is still a black-box approach in the sense that it does not require the determination of organ distribution or give physiological meaning to the pharmacokinetic parameters. On the other hand, the so-called physiological approach (Mordenti, 1986b), whereby complex physiological flow models are solved to predict drug concentrations and tissue distributions, has had considerable success for those drugs where sufficient data exists.

But in many, and perhaps most, applications the physiological model is not practical and the allometric scaling of half-life, clearance and distribution volumes appears to give reliable predictions at low cost. These parameters are, of course, a reflection of the system as a whole, or at least several interacting components. And even though there are numerous differences in the individual metabolic processes of different species (Mellet, 1969; Calabrese, 1986), the integrating parameters appear to average out these differences, at least for many kinds of chemicals.

In a series of papers, Boxenbaum (1982b, 1983, 1986) discussed the evolutionary basis for drug metabolism systems and the between-species similarities that allow for interspecies scaling. In these papers, Boxenbaum has continually stressed that drug metabolism (and, presumably, chemical metabolism in general) can be viewed as an evolutionary probe. He has proposed that the ability to scale-up from one species to another using concepts such as pharmacokinetic time is a reflection of an underlying "pharmacokinetic ground plan" resulting from the "multifactorial expressions from species genomes."

Of course, the bulk of the work by pharmacologists has been focussed on drugs and their therapeutic effects. But there is no reason, in principle, why the same concepts should not work, at least for many cases, in toxicological applications. It is reasonable to assume that for many chemicals the plasma

concentration or, for drugs where protein binding is high, the unbound plasma concentration is correlated with toxic responses. Unless the doses are so large that the kinetics are no longer first-order or other effects occur which invalidate the classical pharmacokinetic models, the same parameters, biological half-life, clearance, distribution volumes, etc., are involved.

While in many cases the results will be very similar if not identical to those obtained using the approaches described in Section 3.4, or using the Kleiber–Brody scaling, there are cases where the results may differ. In those situations where distribution volumes are not proportional to mass or where clearance is not proportional to the two-thirds or three-fourths power of the mass, significant differences can arise. Moreover, as shown by Boxenbaum (1984), the need to use brain weight as well as body weight in scaling some drugs (normally those where low hepatic extraction as opposed to renal elimination is involved) introduces a multivariate aspect to the problem.

Furthermore, the recognition that similar peak plasma concentrations and AUC values give similar responses leads to the result that the dose regimens for small animals must be very different than for humans if they are to give reliable predictions for short-term exposures. This phenomenon has important implications for short-term health advisories used by the US EPA to advise communities that are subjected to acute episodes of drinking water contamination lasting from a few days to a few weeks.

Clearly, the advances in pharmacokinetic interspecies scaling have important implications for interspecies extrapolation of toxicological data and they should be exploited to the fullest extent in planning experiments, interpreting data and developing protocols.

It appears that, for example, in a toxicological evaluation for both short-term and long-term exposures, one of the first steps is to determine the fundamental pharmacokinetic parameters involved in the allometric model as defined by Mordenti (1986b). This should be done for several (Mordenti suggests four) animal species using young adults. Using linear regression analysis, the investigator can determine whether the fundamental parameters such as distribution volumes, half-life and clearance or area under the curve of total or unbound plasma concentration obey allometric equations. If they do obey allometric equations, then there is reason to believe that extrapolations can be made to humans. In some cases, this could be checked by giving small doses to humans to see if the observed human values for the various parameters such as AUC are close to the predicted values.

Short-term toxicological tests should be conducted with the same animal species to determine if there is a consistent relationship between total or unbound concentration in the plasma and the response; that is, does the same concentration elicit the same response (e.g. LD_{50}). In designing these experiments, attention will have to be paid to the fact that animals with faster clearance will generally require more frequent and larger doses.

For chemicals that involve hepatic clearance, it may be necessary to develop multivariate allometric equations using both body weight and brain weight (Boxenbaum and Ronfeld, 1983).

The degree of fit of the animal data to the classical one-, two- or three-compartmental pharmacokinetic models and the allometric equation provides a guide to the appropriateness of the simpler allometric model. If there are indications that non-linearities are present at the dose levels being tested in the animals, then the PB-PK model may be appropriate, although non-linear elimination mechanisms can be used in allometric scaling.

In view of the expense involved in applying the PB-PK models, as well as the relative scarcity of people trained in their use (these models should not be used by the inexperienced), the allometric approach should be tried first.

Specific research programmes could be carried out to provide better guidance. One could focus on developing criteria for when a PB-PK model is needed by determining if there are relatively simple ways of determining when non-linearities (and other factors which invalidate the allometric models) are present. Another could test the allometric and PB-PK models on several compounds representing major classes of chemicals and pharmacokinetic behaviours. A simple experiment would involve checking to see if the same response to various chemicals is elicited by the same plasma concentration. This work would be very similar to the work of Brodie and Reid (1967).

It may be possible to develop some of this information using data from the literature for a few compounds. But the lack of uniformity of the toxicological data developed so far does not lend confidence to the use of existing data for the allometric pharmacokinetic models. At any rate, for most compounds, the requisite data, such as plasma concentrations as a function of time, does not exist.

The type of research described above could, and should, be shared by government and private industry. In many cases, short-term toxicological testing already being carried out by industry on its products as a part of regulatory compliance can provide the type of pharmacokinetic data described here with very little additional cost. Indeed, in the long-run, there is a considerable potential for savings in time and money because most of the data required can be obtained from short-term experiments. Moreover, the accurate prediction of toxicological properties from small animals such as rats and mice may eventually make the use of dogs and other larger animals largely unnecessary, or at least minimal, and thereby avoid some of the present controversy that results from the use of such animals.

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References

Adolph, E. F. (1949). *Science* **109**, 579–585.
Andersen, M. E., Clewell, H. J., III, Gargas, M. L., Smith, F. A. and Reitz, R. H. (1987). *Toxic Appl. Pharmacol.* **87**, 185–205.
Baker, R. J., Kozoll, D. D. and Meyer, K. A. (1957). *Surg. Gynecol. Obst.* **104**, 183–189.
Bertalanffy, L. V. (1957). *Quart. Rev. Biol.* **32**, 217–231.
Bischoff, K. B. (1975). *Cancer Chemother. Rep., Part 1* **59**, 777–793.
Bischoff, K. B. (1986). *Bull. Math. Biol.* **48**, 309–322.
Bischoff, K. B. (1987). *In* "Pharmacokinetics in Risk Assessment: Drinking Water and Health", Vol. 8, pp. 36–61. National Academy Press, Washington, DC.
Bischoff, K. B. and Dedrick, R. L. (1968). *J. Pharm. Sci.* **57**, 1346–1351.
Blum, J. J. (1977). *J. Theor. Biol.* **64**, 599–601.
Boddington, M. J. (1978). *J. Theor. Biol.* **75**, 443–449.
Book, S. A. (1982). *J. Toxicol. Env. Health* **9**, 710–725.
Boxenbaum, H. (1980). *J. Pharmacok. Biopharm.* **8**, 165–176.
Boxenbaum, H. (1982a). *J. Pharmacok. Biopharm.* **10**, 411–426.
Boxenbaum, H. (1982b). *J. Pharmacok. Biopharm.* **10**, 201–227.
Boxenbaum, H. (1983). *Drug Metab. Rev.* **14**, 1057–1097.
Boxenbaum, H. (1984). *Drug Metab. Rev.* **15**, 1071–1121.
Boxenbaum, H. (1986). *J. Pharm. Sci.* **75**, 1053–1062.
Boxenbaum, H. and Fertig, J. B. (1984). *Eur. J. Drug Met. Pharmacokin.* **9**, 177–183.
Boxenbaum, H. and Ronfeld, R. (1983). *Am. J. Physiol.* **245**, R768–774.
Boxenbaum, H. and D'Souza, R. W. (1990). Interspecies pharmacokinetic scaling, biological design and neotony. *In* "Advances in Drug Research" (B. Testa, ed.), Vol. 19, pp. 139–196. Academic Press, London.
Brodie, B. B. (1962). *Clin. Pharmacol. Ther.* **3**, 374–380.
Brodie, B. B. and Reid, W. D. (1967). *Fed. Proc.* **26**, 1062–1070.
Brody, S. (1937). *Growth* **1**, 61–67.
Brody, S. (1945) "Bioenergetics and Growth, with Special Reference to the Efficiency Complex in Domestic Animals". Hafner Press, New York, and Collier MacMillan Publishers, London.
Brody, S. and Proctor, R. C. (1932). Further investigations of surface area in energy metabolism. *Mo. Res. Bull.* **116**, University of Missouri, Columbia, Mo.
Buckingham, E. (1914). *Phys. Rev.* **4**, 345.
Butler, A. M. and Richie, R. H. (1960). *New Engl. J. Med.* **262**, 903–907.
Calabrese, E. J. (1983). "Principles of Animal Extrapolation". John Wiley & Sons, New York.
Calabrese, E. J. (1986). *J. Pharm. Sci.* **75**, 1041–1046.
Calder, W. A., III (1981). *Ann. Rev. Physiol.* **43**, 301–323.
Calder, W. A., III (1984). "Size, Function and Life History". Harvard University Press, Cambridge, MA.

Chappell, W. R. (1984). "Use of Surface Area as the Basis for Non-carcinogenic Risk Assessment". Report to ICAIR for the Office of Drinking Water, U.S. EPA.
Chappell, W. R. (1985). *In* "Trace Substances in Environmental Health" (D. D. Hemphill, ed.), Vol. XIX, pp. 326–337. University of Missouri, Columbia, MO.
Chappell, W. R. (1989). *Risk Anal.* **9**, 13–14.
Chen, H. S. G. and Gross, J. F. (1979). *Cancer Chemother. Pharmacol.* **2**, 85–94.
Collins, J. M., Zaharko, D. S., Dedrick, R. L. and Chabner, B. A. (1986). *Cancer Treatment Rep.* **70**, 73–80.
Crawford, J. D., Terry, M. E. and Rourke, G. M. (1950). *Pediatrics* **5**, 783–796.
Davidson, I. W. F., Parker, J. C. and Beilles, R. P. (1986). *Reg. Toxic. Pharmacol.* **6**, 211–237.
Dedrick, R. L., Bischoff, K. B. and Zaharko, D. S. (1970). *Cancer Chemother. Rep. Part 1* **54**, 95–101.
Derome, J. R. (1977). *Biol.* **65**, 369–378.
Done, A. K. (1964). *Clin. Pharmacol. Ther.* **5**, 432–465.
Dreyer, G. and Walker, E. W. A. (1914). *Ther. Pharmacol. Section. Proc. Roy. Soc. Med.* **7**, 51–70.
D'Souza, R. W., Francis, W. R., Bruce, R. D. and Andersen, M. E. (1987). *In* "Pharmacokinetics in Risk Assessment; Drinking Water and Health", Vol. 8, pp. 286–301. National Academy of Sciences, Washington, D.C.
Economos, A. C. (1977). *Physiologist* **22**, 571–572.
Feldman, H. A. and McMahon, T. A. (1983). *Respiration Physiology* **48**, 149–163.
Forbes, G. B. (1959). *Pediatrics* **23**, 3–5.
Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H. and Skipper, H. E. (1966). *Cancer Chemother. Rep.* **50**, 219–244.
Friis-Hansen, B. (1961). *Pediatrics* **28**, 169–181.
Fujita, M., Iwamoto, J. and Kondo, M. (1966). *Health Phys.* **12**, 1237–1247.
Furchner, J. E. and Richmond, C. R. (1963). *Health Phys.* **9**, 277–282.
Galileo, G. (1637). "Dialogues Concerning Two New Sciences" (H. Crew and A. De Salvio, translators), 1950 edition. Northwestern University Press, Evanston, OH.
Gerlowski, L. E. and Jain, R. K. (1983). *J. Pharm. Sci.* **72**, 1103–1127.
Glantz, S. A., Kernoff, R. and Goldman, R. H. (1976). *Circulation Res.* **39**, 407–414.
Goldsmith, M. A., Slavik, M. and Carter, S. K. (1975). *Cancer Res.* **35**, 1354–1364.
Gould, S. J. (1966). *Biol. Rev.* **41**, 587–649.
Gould, S. J. (1979). *New Scientist*, Feb. 8, 388–389.
Gray, I. F. and Daniel, L. J. (1964). *J. Nutr.* **84**, 31–37.
Griffin, G. E., Abbott, W. E. and Pride, M. P. *et al.* (1945). *Ann. Surg.* **121**, 352–360.
Grollman, A. (1929). *Am. J. Physiol.* **90**, 210–217.
Gunther, B. (1975). *Physiological Rev.* **55**, 659–699.
Gunther, B. and Guerra, E. (1955). *Acta Physiol. Lat. Am.* **5**, 169–186.
Gunther, B. and Guerra, E. (1957). *Acta Physiol. Lat. Am.* **7**, 95–103.
Gunther, B. and Martinoya, C. (1968). *J. Theor. Biol.* **20**, 107–111.
Hahn, L. (1952). *Arch. Dis. Child.* **27**, 43–53.
Haldane, J. B. S. (1928). "On Being the Right Size, Possible Worlds and Other Essays". Chatto and Windus, London.
Harwood, P. D. (1963). *Science* **139**, 685.
Hayssen, V. and Lacy, R. C. (1985). *Comp. Biochem. Physiol.* **81A**, 741–754.
Hemmingsen, A. M. (1960). *Rep. Steno. Mem. Hosp. (Copenhagen),* **9** (Part II), 110 pp.
Heusner, A. A. (1982a). *Respir. Physiol.* **48**, 1–12.
Heusner, A. A. (1982b). *Respir. Physiol.* **48**, 13–25.

Hill, A. V. (1950). *Sci. Progr. London* **38**, 209–230.
Himmelstein, K. J., Lutz, R. J. (1979). *J. Pharmacokin. Biopharm.* **7**, 127–145.
Hoel, D. G., Gaylor, D. W., Kirschstein, R. L., Saffioti, V. and Schneiderman, M. A. (1975). *J. Toxic. Env. Health* **1**, 133–151.
Homan, E. R. (1972). *Cancer Chemother. Rep.* **3**, 13–19.
Huxley, J. S. (1932). "Problems of Relative Growth". Reprinted in 1972 by Dover, New York.
Huxley, J. S. and Teissier, G. (1936). *Nature* **137**, 780–781.
Kleiber, M. (1932). *Hilgardia* **6**, 315–353.
Kleiber, M. (1947). *Physiol. Rev.* **27**, 511–541.
Kleiber, M. (1961). "The Fire of Life". John Wiley, New York.
Kleiber, M. (1967). *Ann. Rev. Physiol.* **29**, 1–20.
Kleiber, M. (1975). *J. Theor. Biol.* **53**, 199–204.
Klotz, U., Antonin, K. H. and Bieck, P. R. (1976). *J. Pharmacol. Exp. Ther.* **199**, 67–73.
Koval'skiy, V. V., Yarovaya, G. A. and Shmavoryan, D. M. (1961). *Zh. Obshch. Biol.* **22**, 179–191.
Krasovskii, G. N. (1976). *Env. Health Persp.* **13**, 51–58.
Lambert, R. and Teissier, G. (1927). *Ann. Physiol.* **3**, 212–246.
Lee, J. F. and Sears, F. W. (1963). *In* "Thermodynamics", pp. 321–324. Addison-Wesley, New York.
Lutz, R. J., Dedrick, R. L. and Zaharko, D. S. (1980). *Pharmacol. Ther.* **11**, 559–592.
Mantel, N. and Schneiderman, M. A. (1975). *Cancer Res.* **35**, 1379–1386.
McMahon, T. (1973). *Science* **179**, 1201–1204.
McMahon, T. A. (1984). "Muscles, Reflexes, and Locomotion". Princeton University Press, Princeton, NJ.
McMahon, T. and Bonner, J. T. (1983). "On Size and Life". Scientific American Books, New York.
McGovern, J. P., Williams, M. G. and Stewart, J. C. (1988). *Drug Metab. Disp.* **16**, 18–22.
McWilliams, P. C., Furchner, J. E. and Richmond, C. R. (1964). *Health Phys.* **10**, 817–822.
Meeh, K. (1979). *Zschr. Biol.* **15**, 425–458.
Mellett, L. B. (1969). *Prog. Drug Res.* **13**, 136–139.
Moore, B. (1909). *Biochem. J.* **4**, 323–330.
Mordenti, J. (1985a). *Antimicrob. Agents Chemother.* **27**, 887–891.
Mordenti, J. (1985b). *J. Pharm. Sci.* **74**, 1097–1099.
Mordenti, J. (1986a). *J. Pharm. Sci.* **75**, 853–857.
Mordenti, J. (1986b). *J. Pharm. Sci.* **75**, 1028–1040.
Mordenti, J. and Chappell, W. R. (1989). The use of interspecies scaling in toxicokinetics. *In* "Toxicokinetics and New Drug Development" (A. Yacobi, J. P. Skelly and V. K. Batra, eds), pp. 42–96. Pergamon Press, New York.
National Academy of Sciences (NAS), Committee on Medical and Biological Effects of Environmental Pollutants (1977). "Nitrogen Oxides". NAS/NRC, Washington, D.C.
National Academy of Sciences (NAS) (1987) "Pharmacokinetics in Risk Assessment, Drinking Water and Health", Vol. 8. NAS, Washington D.C.
National Research Council (NRC), Committee on Animal Nutrition (1935). "Report on the Conference on Energy Metabolism". NRC, Washington, D.C.
Oliver, W. J., Graham, B. D. and Wilson, J. L. (1958). *J. Am. Med. Assoc.* **167**, 1211–1217.
Peters, H. P. (1983). "The Ecological Implications of Body Size". Cambridge University Press, Cambridge.
Pinkel, D. (1958). *Cancer Res.* **18**, 853–856.
Prothero, J. W. (1980). *Comp. Biochem. Physiol.* **67A**, 649–657.
Quinn, G. P., Axelrod, J. and Brodie, B. (1958). *Biochem. Pharmacol.* **1**, 152–159.
Quiring, D. P. (1941). *Growth* **5**, 301–327.

Quiring, D. P. (1944). *In* "Medical Physics" (O. Glasser, ed.), pp. 1490–1494. The Year Book Publishers, Chicago.
Rall, D. P. (1969). *Env. Res.* **2**, 360–367.
Rall, D. P. (1979). *Env. Health Perspectives* **32**, 297–300.
Ramsey, J. C. and Andersen, M. E. (1984). *Toxicol. Appl. Pharmacol.* **73**, 159–175.
Richet, C. (1889). "La Chaleur Animale". Bibliothique Sci. International, Paris.
Richmond, C. R., Furchner, J. E. and Trafton, G. A. (1962a). *Health Phys.* **7**, 219–225.
Richmond, C. R., Furchner, J. E., Trafton, G. A. and Langham, W. H. (1962b). *Health Phys.* **8**, 481–489.
Robertson, D. R. C., Waller, D. G., Renwick, A. G. and George, C. F. (1988). *Br. J. Clin. Pharmacol.* **25**, 297–305.
Rowland, M. (1985). *Pharmacol. Ther.* **29**, 49–68.
Rubner, M. (1883). *Wechsel. Zschr. Biol.*, **19**, 535–562.
Sacher, G. A. (1959). Relation of lifespan to brain weight and body in mammals. *In* "Ciba Foundation Symposium of the Lifespan of Animals" (G. E. W. Wolstenholme and M. O. Connor, eds), Churchill, London, pp. 115–133.
Salvadeo, A., Villa, G. and Bovia, G. (1988). *Int. J. Clin. Pharmacol.* **26**, 98–104.
Sarrus and Rameaux (1838). *Bull. Acad. Roy. Med.* **3**, 1094–1100.
Sawada, Y., Hanano, M., Sugiyama, Y. and Iga, T. (1985). *J. Pharmacokin. Biopharm.* **13**, 477–491.
Schein, P. S., Davis, R. D., Carter, S., Newman, J., Schein, D. R. and Rall, D. P. (1970). *Clin. Pharmacol. Ther.* **11**, 3–40.
Schmidt-Nielsen, K. (1970). *Fed. Proc.*, **29**, 1524–1533.
Schmidt-Nielsen, K. (1984). "Scaling: Why is Animal Size So Important". Cambridge University Press, New York.
Scott, A. K., Webster, J., Petrie, J. C. and Bastain, W. (1988). *Br. J. Clin. Pharmacol.* **25**, 165–168.
Shnider, B. I. (1961). *J. Chron. Dis.* **15**, 229–236.
Shy, C. M. (1973) Human health consequences of nitrogen dioxide: A review. *In* "Proc. Conf. Health Effects of Air Pollutants", pp. 363–405. NAS/NRC, Washington.
Smith, H. W. (1951). "The Kidney: Structure and Function in Health and Disease". Oxford Press, New York.
Snively, W. D. Jr. (1957). *Metabolism* **6**, 70–87.
Sollman, J. (1957). Barium. *In* "A Manual of Pharmacology and its Application to Therapeutics and Toxicology". W. B. Saunders, Philadelphia, pp. 665–667.
Spector, W. S. (1956). "Handbook of Biological Data". W. B. Saunders Co., Philadelphia.
Spells, K. E. (1969). *Resp. Physiol.* **8**, 37–57.
Stahl, W. R. (1963). *Adv. Biol. Med. Phys.* **9**, 355–464.
Stahl, W. R. (1967). *J. Appl. Physiol.* **22**, 453–460.
Sugita, O., Sawada, Y., Sugiyama, Y., Iga, T. and Hanano, M. (1982). *J. Pharmacokin. Biopharm.* **10**, 297–316.
Swabb, E. and Bonner, D. (1983). *J. Pharmacokin. Biopharm.* **11**, 215–223.
Takai, S. and Shiamguchi, S. (1986). *Human Biol.* **58**, 625–638.
Talbot, N. B. and Richie, R. H. (1959) *Pediatrics* **24**, 495–498.
Talbot, N. B., Crawford, J. D. and Butler, A. M. (1953). *New Engl. J. Med.* **248**, 1100–1108.
Thompson, D. W. (1917). "On Growth and Form". Abridged edition (J. T. Bonner, ed.). Cambridge University Press, 1961.
Travis, C. C. and White, R. K. (1988). *Risk Analysis* **8**, 119–125.
Tsongas, T. A., Meglen, R. R., Walravens, P. A. and Chappell, W. R. (1980). *Am. J. Clin. Nutr.* **33**, 1103–1107.
Tsuji, A., Miyamoto, E., Terasaki, T. and Yamana, T. (1979). *Pharmacology* **3**, 116–119.

Tsuji, A., Yoshikawa, T., Nishide, K., Minami, H., Kimura, M., Nakashima, E., Terasaki, T., Miyamoto, E., Nightingale, C. and Yamana, T. (1983). *J. Pharm. Sci.* **72**, 1346–1351.
Tsuji, A., Nishide, K., Minami, H., Nakashima, E., Terasaki, T. and Ymana, T. (1985). *Drug Metab. Disp.* **13**, 729–739.
Underwood, E. J. (1976). *In* "Molybdenum in the Environment" (W. R. Chappell and K. K. Petersen, eds), Vol. 1. pp. 9–32. Marcel Dekker, Inc., New York.
Venugopal, B. and Luckey, T. D. (1978). "Metal Toxicity in Mammals", Vol. 2. Plenum Press, New York.
Voit, E. (1901). *Ztschr. Biol.* **41**, 113–154.
Wagner, J. G., Northam, J. I., Alway, C. D. and Carpenter, O. S. (1965). *Nature* **207**, 1301–1302.
West, L. J. and Pierce, C. M. (1962). *Science* **138**, 1100–1103.
Wieser, W. (1984). *Resp. Physiol.* **55**, 1–9.
Weiss, M., Sziegoleit, W. and Forster, W. (1977). *Int. J. Clin. Pharmacol.* **15**, 572–575.
Yates, F. E. and Kugler, P. N. (1986). *J. Pharm. Sci.* **75**, 1019–1027.
Zar, J. H. (1968). *BioScience* **18**, 1118–1120.

Tissue Binding versus Plasma Binding of Drugs: General Principles and Pharmacokinetic Consequences

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Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

U.S. Department of Health and Human Services
Food and Drug Administration
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July 2005
Pharmacology and Toxicology

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Contains Nonbinding Recommendations

Guidance for Industry[1] Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance outlines a process (algorithm) and vocabulary for deriving the maximum recommended starting dose (MRSD) for *first-in-human* clinical trials of new molecular entities in adult healthy volunteers, and recommends a standardized process by which the MRSD can be selected. The purpose of this process is to ensure the safety of the human volunteers.

The goals of this guidance are to: (1) establish a consistent terminology for discussing the starting dose; (2) provide common conversion factors for deriving a human equivalent dose (HED); and (3) delineate a strategy for selecting the MRSD for adult healthy volunteers, regardless of the projected clinical use. This process is depicted in a flow chart that presents the decisions and calculations used to generate the MRSD from animal data (see Appendix E).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

[1] This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

II. BACKGROUND

The process identified in this guidance pertains to determining the MRSD for adult healthy subjects when beginning a clinical investigation of any new drug or biological therapeutic that has been studied in animals. This guidance is not pertinent to endogenous hormones and proteins (e.g., recombinant clotting factors) used at physiologic concentrations or prophylactic vaccines. The process outlined in this guidance pertains primarily to drug products for which systemic exposure is intended; it does not address dose escalation or maximum allowable doses in clinical trials.

Although the process outlined in this guidance uses administered doses, observed toxicities, and an algorithmic approach to calculate the MRSD, an alternative approach could be proposed that places primary emphasis on animal pharmacokinetics and modeling rather than dose (Mahmood et al. 2003; Reigner and Blesch 2002). In a limited number of cases, animal pharmacokinetic data can be useful in determining initial clinical doses.[2] However, in the majority of investigational new drug applications (INDs), animal data are not available in sufficient detail to construct a scientifically valid, pharmacokinetic model whose aim is to accurately project an MRSD.

Toxicity should be avoided at the initial clinical dose. However, doses should be chosen that allow reasonably rapid attainment of the phase 1 trial objectives (e.g., assessment of the therapeutic's tolerability, pharmacodynamic or pharmacokinetic profile). All of the relevant preclinical data, including information on the pharmacologically active dose, the full toxicologic profile of the compound, and the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the therapeutic, should be considered when determining the MRSD. Starting with doses lower than the MRSD is always an option and can be particularly appropriate to meet some clinical trial objectives.

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[2] If the parent drug is measured in the plasma at multiple times and is within the range of toxic exposures for two or more animal species, it may be possible to develop a pharmacokinetic model predicting human doses and concentrations and to draw inferences about safe human plasma levels in the absence of prior human data. Although quantitative modeling for this purpose may be straightforward, the following points suggest this approach can present a number of difficulties when estimating a safe starting dose. Generally, at the time of IND initiation, there are a number of unknowns regarding animal toxicity and comparability of human and animal pharmacokinetics and metabolism: (1) human bioavailability and metabolism may differ significantly from that of animals; (2) mechanisms of toxicity may not be known (e.g., toxic accumulation in a peripheral compartment); and/or (3) toxicity may be due to an unidentified metabolite, not the parent drug. Therefore, relying on pharmacokinetic models (based on the parent drug in plasma) to gauge starting doses would require multiple untested assumptions. Modeling can be used with greatest validity to estimate human starting doses in special cases where few underlying assumptions would be necessary. Such cases are exemplified by large molecular weight proteins (e.g., humanized monoclonal antibodies) that are intravenously administered, are removed from circulation by endocytosis rather than metabolism, have immediate and detectable effects on blood cells, and have a volume of distribution limited to the plasma volume. In these cases, allometric, pharmacokinetic, and pharmacodynamic models have been useful in identifying the human mg/kg dose that would be predicted to correlate with safe drug plasma levels in nonhuman primates. Even in these cases, uncertainties (such as differences between human and animal receptor sensitivity or density) have been shown to affect human pharmacologic or toxicologic outcomes, and the use of safety factors as described in this guidance is still warranted.

The remainder of this guidance focuses on the recommended algorithmic process for starting dose extrapolation from animals to humans based on administered doses, since this method will likely be useful for the majority of INDs seeking to investigate new drugs in healthy volunteers. Some classes of drugs (e.g., many cytotoxic or biological agents) are commonly introduced into initial clinical trials in patient volunteers rather than healthy volunteers. Typically, patients are used instead of healthy volunteers when a drug is suspected or known to be unavoidably toxic. This guidance does not address starting doses in patients. However, many principles and some approaches recommended here may be applicable to designing such trials.

III. OVERVIEW OF THE ALGORITHM

The recommended process for selecting the MRSD is presented in Appendix E and described in this section. The major elements (i.e., the determination of the no observed adverse effect levels (NOAELs) in the tested animal species, conversion of NOAELs to HED, selection of the most appropriate animal species, and application of a safety factor) are all discussed in greater detail in subsequent sections. Situations are also discussed in which the algorithm should be modified. The algorithm is intended to be used for systemically administered therapeutics. Topical, intranasal, intratissue, and compartmental administration routes and depot formulations can have additional considerations, but similar principles should apply.

The process of calculating the MRSD should begin after the toxicity data have been analyzed. Although only the NOAEL should be used directly in the algorithm for calculating an MRSD, other data (exposure/toxicity relationships, pharmacologic data, or prior clinical experience with related drugs) can affect the choice of most appropriate species, scaling, and safety factors.

The NOAEL for each species tested should be identified, and then converted to the HED using appropriate scaling factors. For most systemically administered therapeutics, this conversion should be based on the normalization of doses to body surface area. Although body surface area conversion is the standard way to approximate equivalent exposure if no further information is available, in some cases extrapolating doses based on other parameters may be more appropriate. This decision should be based on the data available for the individual case. The body surface area normalization and the extrapolation of the animal dose to human dose should be done in one step by dividing the NOAEL in each of the animal species studied by the appropriate body surface area conversion factor (BSA-CF). This conversion factor is a unitless number that converts mg/kg dose for each animal species to the mg/kg dose in humans, which is equivalent to the animal's NOAEL on a mg/m^2 basis. The resulting figure is called a human equivalent dose (HED). The species that generates the lowest HED is called the most sensitive species.

When information indicates that a particular species is more relevant for assessing human risk (and deemed the *most appropriate species*), the HED for that species may be used in subsequent calculations, regardless of whether this species is the most sensitive. This situation is more applicable to biologic therapies, many of which have high selectivity for binding to human target

proteins and limited reactivity in species commonly used for toxicity testing. In such cases, in vitro binding and functional studies should be conducted to select an appropriate, relevant species before toxicity studies are designed (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details[3]). (However, if serious toxicities are observed in an animal species considered less relevant, those toxicities should be taken into consideration in determining the species to be used to calculate an HED. For example, in one particular case, dog was selected as the animal species used for calculation of an HED because of unmonitorable cardiac lesions, even though the rat was considered the most relevant species based on pharmacological activity data.) Additionally, a species might be considered an inappropriate toxicity model for a given drug if the dose-limiting toxicity in that species was concluded to be of limited value for human risk assessment, based on historical comparisons of toxicities in the animal species to those in humans across a therapeutic class (i.e., the dose-limiting toxicity is species-specific). In this case, data from that species should not be used to derive the HED. Without any additional information to guide the choice of the most appropriate species for assessing human risk, the most sensitive species is designated the *most appropriate*, because using the lowest HED would generate the most conservative starting dose.

A safety factor should then be applied to the HED to increase assurance that the first dose in humans will not cause adverse effects. The use of the safety factor should be based on the possibility that humans may be more sensitive to the toxic effects of a therapeutic agent than predicted by the animal models, that bioavailability may vary across species, and that the models tested do not evaluate all possible human toxicities. For example, ocular disturbances or pain (e.g., severe headaches) in humans can be significant dose-limiting toxicities that may go undetected in animal studies.

In general, one should consider using a safety factor of at least 10. The MRSD should be obtained by dividing the HED by the safety factor. Safety concerns or design shortcomings noted in animal studies may increase the safety factor, and thus reduce the MRSD further. Alternatively, information about the pharmacologic class (well-characterized classes of therapeutics with extensive human clinical and preclinical experience) may allay concerns and form the basis for reducing the magnitude of the default safety factor and increasing the MRSD. Although a dose lower than the MRSD can be used as the actual starting dose, the process described in this guidance will derive the maximum recommended starting dose. This algorithm generates an MRSD in units of mg/kg, a common method of dosing used in phase 1 trials, but the equations and conversion factors provided in this guidance (Table 1, second column) can be used to generate final dosing units in the mg/m^2 form if desired.

As previously stated, for purposes of initial clinical trials in adult healthy volunteers, the HED should ordinarily be calculated from the animal NOAEL. If the HED is based on an alternative index of effect, such as the pharmacologically active dose (PAD), this exception should be prominently stipulated in descriptions of starting dose calculations.

[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

The remainder of this guidance provides a description of the individual steps in the recommended process and the reasoning behind each step.

IV. STEP 1: NO OBSERVED ADVERSE EFFECT LEVEL DETERMINATION

The first step in determining the MRSD is to review and evaluate the available animal data so that a NOAEL can be determined for each study. Several definitions of NOAEL exist, but for selecting a starting dose, the following is used: the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group. In this context, adverse effects that are biologically significant (even if they are not statistically significant) should be considered in the determination of the NOAEL. The NOAEL is a generally accepted benchmark for safety when derived from appropriate animal studies and can serve as the starting point for determining a reasonably safe starting dose of a new therapeutic in healthy (or asymptomatic) human volunteers.

The NOAEL is not the same as the *no observed effect level (NOEL)*, which refers to any effect, not just an adverse one, although in some cases the two might be identical. The definition of the NOAEL, in contrast to that of the NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern. The NOAEL should also not be confused with *lowest observed adverse effect level (LOAEL)* or *maximum tolerated dose (MTD)*. Both of the latter concepts are based on findings of adverse effects and are not generally used as benchmarks for establishing safe starting doses in adult healthy volunteers. (The term *level* refers to dose or dosage, generally expressed as mg/kg or mg/kg/day.)

Initial IND submissions for first-in-human studies by definition lack in vivo human data or formal allometric comparison of pharmacokinetics. Measurements of systemic levels or exposure (i.e., AUC or C_{max}) cannot be employed for setting a safe starting dose in humans, and it is critical to rely on dose and observed toxic response data from adequate and well-conducted toxicology studies. However, there are cases where nonclinical data on bioavailability, metabolite profile, and plasma drug levels associated with toxicity may influence the choice of the NOAEL. One such case is when saturation of drug absorption occurs at a dose that produces no toxicity. In this instance, the lowest saturating dose, not the highest (nontoxic) dose, should be used for calculating the HED.

There are essentially three types of findings in nonclinical toxicology studies that can be used to determine the NOAEL: (1) overt toxicity (e.g., clinical signs, macro- and microscopic lesions); (2) surrogate markers of toxicity (e.g., serum liver enzyme levels); and (3) exaggerated pharmacodynamic effects. Although the nature and extent of adverse effects can vary greatly with different types of therapeutics, and it is anticipated that in many instances, experts will disagree on the characterization of effects as being adverse or not, the use of NOAEL as a benchmark for dose-setting in healthy volunteers should be acceptable to all responsible investigators. As a general rule, an adverse effect observed in nonclinical toxicology studies

used to define a NOAEL for the purpose of dose-setting should be based on an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase 1 clinical trial conducted in adult healthy volunteers.

V. STEP 2: HUMAN EQUIVALENT DOSE CALCULATION

A. Conversion Based on Body Surface Area

After the NOAELs in the relevant animal studies have been determined, they are converted to HEDs. A decision should be made regarding the most appropriate method for extrapolating the animal dose to the equivalent human dose. Toxic endpoints for therapeutics administered systemically to animals, such as the MTD, are usually assumed to scale well between species when doses are normalized to body surface area (i.e., mg/m^2) (EPA 1992; Lowe and Davis 1998). The basis for this assumption lies primarily with the work of Freireich et al. (1966) and Schein et al. (1970). These investigators reported that, for antineoplastic drugs, doses lethal to 10 percent of rodents (LD_{10}s) and MTDs in nonrodents both correlated with the human MTD when the doses were normalized to the same administration schedule and expressed as mg/m^2. Despite the subsequent analyses showing that the MTDs for this set of drugs scale best between species when doses are normalized to $W^{0.75}$ rather than $W^{0.67}$ (inherent in body surface area normalization) (Travis and White 1988; Watanabe et al. 1992), normalization to body surface area has remained a widespread practice for estimating an HED based on an animal dose.

An analysis of the affect of the allometric exponent on the conversion of an animal dose to the HED was conducted (see Appendix A). Based on this analysis and on the fact that correcting for body surface area increases clinical trial safety by resulting in a more conservative starting dose estimate, it was concluded that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., $W^{0.67}$) should be maintained for selecting starting doses for initial studies in adult healthy volunteers. Nonetheless, use of a different dose normalization approach, such as directly equating the human dose to the NOAEL in mg/kg, may be appropriate in some circumstances. Deviations from the body surface area approach, when describing the conversion of animal dose to HED, should be justified. The basis for justifying direct mg/kg conversion and examples in which other normalization methods are appropriate are described in the following subsection.

Although normalization to body surface area is an appropriate method for extrapolating doses between species, consistent factors for converting doses from mg/kg to mg/m^2 have not always been used. Given that body surface area normalization provides a reasonable approach for estimating an HED, the factors used for converting doses for each species should be standardized. Since body surface area varies with $W^{0.67}$, the conversion factors are dependent on the weight of the animals in the studies. However, analyses conducted to address the effect of body weight on the actual BSA-CF demonstrated that a standard factor provides a reasonable estimate of the HED over a broad range of human and animal weights (see Appendix B). The conversion factors and divisors shown in Table 1 are therefore recommended as the standard

values to be used for interspecies dose conversions for NOAELs. (These factors may also be applied when comparing safety margins for other toxicity endpoints (e.g., reproductive toxicity and carcinogenicity) when other data for comparison (i.e., AUCs) are unavailable or are otherwise inappropriate for comparison.)

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m^2, Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

[a] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[b] This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

[c] For example, cynomolgus, rhesus, and stumptail.

B. Basis for Using mg/kg Conversions

The factors in Table 1 for scaling animal NOAEL to HEDs are based on the assumption that doses scale 1:1 between species when normalized to body surface area. However, there are occasions for which scaling based on body weight (i.e., setting the HED (mg/kg) = NOAEL (mg/kg)) may be more appropriate. To consider mg/kg scaling for a therapeutic, the available data should show that the NOAEL occurs at a similar mg/kg dose across species. The following circumstances should exist before extrapolating to the HED on a mg/kg basis rather than using

the mg/m^2 approach. Note that mg/kg scaling will give a twelve-, six-, and twofold higher HED than the default mg/m^2 approach for mice, rats, and dogs, respectively. If these circumstances do not exist, the mg/m^2 scaling approach for determining the HED should be followed as it will lead to a safer MRSD.

1. NOAELs occur at a similar mg/kg dose across test species (for the studies with a given dosing regimen relevant to the proposed initial clinical trial). (However, it should be noted that similar NOAELs on a mg/kg basis can be obtained across species because of differences in bioavailability alone.)

2. If only two NOAELs from toxicology studies in separate species are available, one of the following should also be true:

   - The therapeutic is administered orally and the dose is limited by local toxicities. Gastrointestinal (GI) compartment weight scales by $W^{0.94}$ (Mordenti 1986). GI volume determines the concentration of the therapeutic in the GI tract. It is then reasonable that the toxicity of the therapeutic would scale by mg/kg ($W^{1.0}$).

   - The toxicity in humans (for a particular class) is dependent on an exposure parameter that is highly correlated across species with dose on a mg/kg basis. For example, complement activation by systemically administered antisense oligonucleotides in humans is believed to be dependent upon C_{max} (Geary et al. 1997). For some antisense drugs, the C_{max} correlates across nonclinical species with mg/kg dose and in such instances mg/kg scaling would be justified.

   - Other pharmacologic and toxicologic endpoints also scale between species by mg/kg for the therapeutic. Examples of such endpoints include the MTD, lowest lethal dose, and the pharmacologically active dose.

   - There is a robust correlation between plasma drug levels (C_{max} and AUC) and dose in mg/kg.

C. Other Exceptions to mg/m^2 Scaling Between Species

Scaling between species based on mg/m^2 is not recommended for the following categories of therapeutics:

1. Therapeutics administered by alternative routes (e.g., topical, intranasal, subcutaneous, intramuscular) for which the dose is limited by local toxicities. Such therapeutics should be normalized to concentration (e.g., mg/area of application) or amount of drug (mg) at the application site.

2. Therapeutics administered into anatomical compartments that have little subsequent distribution outside of the compartment. Examples are intrathecal, intravesical,

intraocular, or intrapleural administration. Such therapeutics should be normalized between species according to the compartmental volumes and concentrations of the therapeutic.

3. Proteins administered intravascularly with $M_r > 100,000$ daltons. Such therapeutics should be normalized to mg/kg.

VI. STEP 3: MOST APPROPRIATE SPECIES SELECTION

After the HEDs have been determined from the NOAELs from all toxicology studies relevant to the proposed human trial, the next step is to pick one HED for subsequent derivation of the MRSD. This HED should be chosen from the most appropriate species. In the absence of data on species relevance, a default position is that the most appropriate species for deriving the MRSD for a trial in adult healthy volunteers is the most sensitive species (i.e., the species in which the lowest HED can be identified).

Factors that could influence the choice of the most appropriate species rather than the default to the most sensitive species include: (1) differences in the absorption, distribution, metabolism, and excretion (ADME) of the therapeutic between the species, and (2) class experience that may indicate a particular animal model is more predictive of human toxicity. Selection of the most appropriate species for certain biological products (e.g., human proteins) involves consideration of various factors unique to these products. Factors such as whether an animal species expresses relevant receptors or epitopes may affect species selection (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details).

When determining the MRSD for the first dose of a new therapeutic in humans, absorption, distribution, and elimination parameters will not be known for humans. Comparative metabolism data, however, might be available based on in vitro studies. These data are particularly relevant when there are marked differences in both the in vivo metabolite profiles and HEDs in animals. Class experience implies that previous studies have demonstrated that a particular animal model is more appropriate for the assessment of safety for a particular class of therapeutics. For example, in the nonclinical safety assessment of the phosphorothioate antisense drugs, the monkey is considered the most appropriate species because monkeys experience the same dose limiting toxicity as humans (e.g., complement activation) whereas rodents do not. For this class of therapeutics, the MRSD would usually be based on the HED for the NOAEL in monkeys regardless of whether it was lower than that in rodents, unless unique dose limiting toxicities were observed with the new antisense compound in the rodent species.

VII. STEP 4: APPLICATION OF SAFETY FACTOR

Once the HED of the NOAEL in the most appropriate species has been determined, a safety factor should then be applied to provide a margin of safety for protection of human subjects receiving the initial clinical dose. This safety factor allows for variability in extrapolating from

animal toxicity studies to studies in humans resulting from: (1) uncertainties due to enhanced sensitivity to pharmacologic activity in humans versus animals; (2) difficulties in detecting certain toxicities in animals (e.g., headache, myalgias, mental disturbances); (3) differences in receptor densities or affinities; (4) unexpected toxicities; and (5) interspecies differences in ADME of the therapeutic. These differences can be accommodated by lowering the human starting dose from the HED of the selected species NOAEL.

In practice, the MRSD for the clinical trial should be determined by dividing the HED derived from the animal NOAEL by the safety factor. The default safety factor that should normally be used is 10. This is a historically accepted value, but, as described below, should be evaluated based on available information.

A safety factor of 10 may not be appropriate for all cases. The safety factor should be raised when there is reason for increased concern, and lowered when concern is reduced because of available data that provide added assurance of safety. This can be visualized as a sliding scale, balancing findings that mitigate the concern for harm to healthy volunteers with those that suggest greater concern is warranted. The extent of the increase or decrease is largely a matter of judgment, using the available information. It is incumbent on the evaluator to clearly explain the reasoning behind the applied safety factor when it differs from the default value of 10, particularly if it is less than 10.

A. Increasing the Safety Factor

The following considerations indicate a safety concern that might warrant increasing the safety factor. In these circumstances, the MRSD would be calculated by dividing the HED by a safety factor that is greater than 10. If any of the following concerns are defined in review of the nonclinical safety database, an increase in the safety factor may be called for. If multiple concerns are identified, the safety factor should be increased accordingly.

- **Steep dose response curve.** A steep dose response curve for significant toxicities in the most appropriate species or in multiple species may indicate a greater risk to humans.

- **Severe toxicities.** Qualitatively severe toxicities or damage to an organ system (e.g., central nervous system (CNS)) indicate increased risk to humans.

- **Nonmonitorable toxicity.** Nonmonitorable toxicities may include histopathologic changes in animals that are not readily monitored by clinical pathology markers.

- **Toxicities without premonitory signs.** If the onset of significant toxicities is not reliably associated with premonitory signs in animals, it may be difficult to know when toxic doses are approached in human trials.

- **Variable bioavailability.** Widely divergent or poor bioavailability in the several animal species, or poor bioavailability in the test species used to derive the HED, suggest a greater possibility for underestimating the toxicity in humans.

- **Irreversible toxicity.** Irreversible toxicities in animals suggest the possibility of permanent injury in human trial participants.

- **Unexplained mortality.** Mortality that is not predicted by other parameters raises the level of concern.

- **Large variability in doses or plasma drug levels eliciting effect.** When doses or exposure levels that produce a toxic effect differ greatly across species or among individual animals of a species, the ability to predict a toxic dose in humans is reduced and a greater safety factor may be needed.

- **Nonlinear pharmacokinetics.** When plasma drug levels do not increase in a dose-related manner, the ability to predict toxicity in humans in relation to dose is reduced and a greater safety factor may be needed.

- **Inadequate dose-response data.** Poor study design (e.g., few dose levels, wide dosing intervals) or large differences in responses among animals within dosing groups may make it difficult to characterize the dose-response curve.

- **Novel therapeutic targets.** Therapeutic targets that have not been previously clinically evaluated may increase the uncertainty of relying on the nonclinical data to support a safe starting dose in humans.

- **Animal models with limited utility.** Some classes of therapeutic biologics may have very limited interspecies cross-reactivity or pronounced immunogenicity, or may work by mechanisms that are not known to be conserved between (nonhuman) animals and humans; in these cases, safety data from any animal studies may be very limited in scope and interpretability.

B. Decreasing the Safety Factor

Safety factors of less than 10 may be appropriate under some conditions. The toxicologic testing in these cases should be of the highest caliber in both conduct and design. Most of the time, candidate therapeutics for this approach would be members of a well-characterized class. Within the class, the therapeutics should be administered by the same route, schedule, and duration of administration; should have a similar metabolic profile and bioavailability; and should have similar toxicity profiles across all the species tested including humans. A smaller safety factor might also be used when toxicities produced by the therapeutic are easily monitored, reversible, predictable, and exhibit a moderate-to-shallow dose-response relationship with toxicities that are

consistent across the tested species (both qualitatively and with respect to appropriately scaled dose and exposure).

A safety factor smaller than 10 could be justified when the NOAEL was determined based on toxicity studies of longer duration compared to the proposed clinical schedule in healthy volunteers. In this case, a greater margin of safety should be built into the NOAEL, as it was associated with a longer duration of exposure than that proposed in the clinical setting. This assumes that toxicities are cumulative, are not associated with acute peaks in therapeutic concentration (e.g., hypotension), and did not occur early in the repeat dose study.

VIII. STEP 5: CONSIDERATION OF THE PHARMACOLOGICALLY ACTIVE DOSE

Selection of a PAD depends upon many factors and differs markedly among pharmacological drug classes and clinical indications; therefore, selection of a PAD is beyond the scope of this guidance. However, once the MRSD has been determined, it may be of value to compare it to the PAD derived from appropriate pharmacodynamic models. If the PAD is from an in vivo study, an HED can be derived from a PAD estimate by using a BSA-CF. This HED value should be compared directly to the MRSD. If this *pharmacologic* HED is lower than the MRSD, it may be appropriate to decrease the clinical starting dose for pragmatic or scientific reasons. Additionally, for certain classes of drugs or biologics (e.g., vasodilators, anticoagulants, monoclonal antibodies, or growth factors), toxicity may arise from *exaggerated pharmacologic* effects. The PAD in these cases may be a more sensitive indicator of potential toxicity than the NOAEL and might therefore warrant lowering the MRSD.

IX. SUMMARY

A strategy has been proposed to determine the maximum recommended starting dose for clinical trials of new therapeutics in adult healthy volunteers. In summary, usually NOAELs from the relevant animal studies should be converted to the HEDs using the standard factors presented in Table 1. Using sound scientific judgment, a safety factor should be applied to the HED from the most appropriate species to arrive at the MRSD. This process is meant to define the upper limit of recommended starting doses and, in general, lower starting doses can be appropriate. The process described in this guidance should foster consistency among sponsors and Agency reviewers.

REFERENCES

Boxenbaum, H and C DiLea, 1995, First-Time-in-Human Dose Selection: Allometric Thoughts and Perspectives, Journal of Clinical Pharmacology, 35:957-966.

Burtles, SS, DR Newell, REC Henrar, and TA Connors, 1995, Revisions of General Guidelines for the Preclinical Toxicology of New Cytotoxic Anticancer Agents in Europe, European Journal of Cancer, 31A:408-410.

Contrera, JF, AC Jacobs, RP Hullahalli, M Mehta, WJ Schmidt, and JA DeGeorge, 1995, Systemic Exposure — Based Alternative to the Maximum Tolerated Dose for Carcinogenicity Studies of Human Therapeutics, Journal of American College of Toxicology, 14:1-10.

EPA, 1992, A Cross-Species Scaling Factor for Carcinogen Risk Assessment Based on Equivalence of $Mg/Kg^{0.75}$/Day, Federal Register, 57:24152-24173.

Freireich, EJ, EA Gehan, DP Rall, LH Schmidt, and HE Skipper, 1966, Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey, and Man, Cancer Chemotherapy Reports, 50:219-244.

Geary, RS, JM Leeds, SP Henry, DK Monteith, and AA Levin, 1997, Antisense Oligonucleotide Inhibitors for the Treatment of Cancer: 1. QUESTION Pharmacokinetic Properties of Phosphorothioate Oligodeoxynucleotides, Anti-Cancer Drug Design, 12:383-393.

Lowe, MC and RD Davis, 1998, The Current Toxicology Protocol of the National Cancer Institute, in K Hellman and SK Carter (eds.), Fundamentals of Cancer Chemotherapy, pp. 228-235, New York: McGraw Hill.

Mahmood, I, MD Green, and JE Fisher, 2003, Selection of the First-Time Dose in Humans: Comparison of Different Approaches Based on Interspecies Scaling of Clearance, 43(7):692-697.

Mordenti, J, 1986, Man Vs. Beast: Pharmacokinetic Scaling in Mammals, Journal of Pharmaceutical Sciences, 75:1028-1040.

Reigner, BG and KS Blesch, 2002, Estimating the Starting Dose for Entry into Humans: Principles and Practice, European Journal of Clinical Pharmacology, 57:835-845.

Schein, PS, RD Davis, S Carter, J Newman, DR Schein, and DP Rall, 1970, The Evaluation of Anticancer Drugs in Dogs and Monkeys for the Prediction of Qualitative Toxicities in Man, Clinical Pharmacology and Therapeutics, 11:3-40.

Spector, WS (ed.), 1956, Handbook of Biological Data, pp. 175, Philadelphia: W.B. Saunders Co.

Stahl, WR, 1967, Scaling of Respiratory Variables in Mammals, Journal of Applied Physiology, 22:453-460.

Travis, CC and RK White, 1988, Interspecies Scaling of Toxicity Data, Risk Analysis, 8:119-125.

Watanabe, K, FY Bois, and L Zeise, 1992, Interspecies Extrapolation: A Reexamination of Acute Toxicity Data, Risk Analysis, 12:301-310.

International Conference on Harmonisation Guidances

ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*

ICH guidance for industry *S3A Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies*

ICH guidance for industry *M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*

GLOSSARY

b: Allometric exponent

Body surface area conversion factor (BSA-CF): A factor that converts a dose (mg/kg) in an animal species to the equivalent dose in humans (also known as the *human equivalent dose*), based on differences in body surface area. A BSA-CF is the ratio of the body surface areas in the tested species to that of an average human.

Human equivalent dose (HED): A dose in humans anticipated to provide the same degree of effect as that observed in animals at a given dose. In this guidance, as in many communications from sponsors, the term HED is usually used to refer to the human equivalent dose of the NOAEL. When reference is made to the human equivalent of a dose other than the NOAEL (e.g., the PAD), sponsors should explicitly and prominently note this usage.

K: A dimensionless factor that adjusts for differences in the surface area to weight ratio of species because of their different body shapes.

k_m: Factor for converting mg/kg dose to mg/m^2 dose

Lowest observed adverse effect level (LOAEL): The lowest dose tested in an animal species with adverse effects.

Maximum recommended starting dose (MRSD): The highest dose recommended as the initial dose in a clinical trial. In clinical trials of adult healthy volunteers, the MRSD is predicted to cause no adverse reactions. The units of the dose (e.g., mg/kg or mg/m^2) may vary depending on practices employed in the area being investigated.

Maximum tolerated dose (MTD): In a toxicity study, the highest dose that does not produce unacceptable toxicity.

No observed adverse effect level (NOAEL): The highest dose tested in an animal species that does not produce a significant increase in adverse effects in comparison to the control group. Adverse effects that are biologically significant, even if not statistically significant, should be considered in determining an NOAEL.

No observed effect level (NOEL): The highest dose tested in an animal species with no detected effects.

Pharmacologically active dose (PAD): The lowest dose tested in an animal species with the intended pharmacologic activity.

Safety factor (SF): A number by which the HED is divided to introduce a margin of safety between the HED and the *maximum recommended starting dose*.

W: Body weight in kg

APPENDIX A:
Analysis of Allometric Exponent on HED Calculations

An analysis was conducted to determine the effect of the allometric exponent on the conversion of an animal dose to the HED. One can derive the following equation (see Appendix C) for converting animal doses to the HED based on body weights and the allometric exponent (b):

$$HED = \text{animal NOAEL} \times (W_{animal}/W_{human})^{(1-b)}$$

Conventionally, for a mg/m^2 normalization *b* would be 0.67, but a number of studies (including the original Freireich data) have shown that MTDs scale best across species when b = 0.75. The Interagency Pharmacokinetics Group has recommended that $W^{0.75}$ be used for interspecies extrapolation of doses in carcinogenicity studies (EPA 1992). There are no data, however, to indicate the optimal method for converting NOAELs to HEDs. Conversion factors were calculated over a range of animal and human weights using $(W_{animal}/W_{human})^{0.33}$ or $(W_{animal}/W_{human})^{0.25}$ to assess the effect on starting dose selection of using b = 0.75 instead of b = 0.67. The results are shown in Table 2. Using an allometric exponent of 0.75 had a big effect on the conversion factor for the smaller species mice and rats. Nonetheless, mice are not commonly used for toxicology studies to support the first-in-human clinical trials. In addition, there is evidence that the area under the plasma concentration versus time curves in rats and humans correlates reasonably well when doses are normalized to mg/m^2 (Contrera et al. 1995). We conclude that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., b = 0.67) should be maintained for selecting starting doses for initial studies in healthy volunteers since: (1) mg/m^2 normalization is widely used throughout the toxicology and pharmacokinetic research communities; (2) mg/m^2 normalization provides a more conservative conversion; (3) there are no data to suggest a superior method for converting NOAELs; and (4) CDER has significant experience in establishing safe starting doses based on mg/m^2, and it is readily calculated.

Table 2: Effect of Allometric Exponent on Conversion Factor[a]

Species	Weight Range[b] (kg)	Conversion Factors[c]			Ratio of 0.75 to 0.67
		Standard	b = 0.67	b = 0.75	
Mouse	0.018-0.033	0.081	0.075	0.141	1.88
Rat	0.09-0.40	0.162	0.156	0.245	1.57
Rabbit	1.5-3	0.324	0.33	0.43	1.30
Monkey	1.5-4	0.324	0.37	0.47	1.27
Dog	6.5-13.0	0.541	0.53	0.62	1.17

[a] conversion factor = $(W_{animal}/W_{human})^{(1-b)}$

[b] human weight range used was 50-80 kg (110-176 lb)

[c] mean conversion factor calculated across entire animal weight range and human weight range

The following summarizes the analysis of the effects of the allometric exponent on HED calculations:

- Changing the allometric exponent from 0.67 to 0.75 had a big effect on the conversion factor for the smaller rodent species; for mice the conversion factors differed by a factor of almost 2.

- Converting doses based on an exponent of 0.75 would lead to higher, more aggressive and potentially more toxic starting doses.

- The limited data available suggest that the most accurate allometric exponent for normalizing MTDs of antineoplastic agents for interspecies extrapolation is b = 0.75, but there are no data to indicate the optimal normalization method for interspecies extrapolation of NOAELs in a broad range of therapeutic classes. Using mg/m^2 is widely adopted throughout the drug development community.

- Unless evidence is provided to the contrary, HED calculations should be based on b = 0.67 (i.e., the standard conversions based on mg/m^2 relationships).

- There was no notable effect of body weight on calculation of the HED within the weight ranges examined.

APPENDIX B:
Analysis of Body Weight Effects on HED Calculations

Accurate conversion of a mg/kg dose to a mg/m^2 dose depends on the actual weight (and surface area) of the test species. A popular formula for converting doses is:

(i) $mg/m^2 = k_m \times mg/kg$

where $k_m = 100/K \times W^{0.33}$ where K is a value unique to each species (Freireich et al. 1966)

or $k_m = 9.09 \times W^{0.35}$ where a K value unique to each species is not needed (Boxenbaum and DiLea 1995; Burtles et al. 1995; Stahl 1956).

The k_m value is not truly constant for any species, but increases within a species as body weight increases. The increase is not linear, but increases approximately proportional to $W^{2/3}$. For example, the k_m value in rats varies from 5.2 for a 100 g rat to 7.0 for a 250 g rat. Strictly speaking, the k_m value of 6 applies only to rats at the *reference weight* of 150 g. For standardization and practical purposes, a fixed k_m factor for each species is preferred. An analysis was undertaken to determine the effect of different body weights within a species on the conversion of an animal dose to the HED using k_m factors. The k_m factor was calculated for a range of body weights using $k_m = 100/K \times W^{0.33}$. In Table 3, a working weight range is shown next to the reference body weight. This is the range within which the HED calculated by using the standard k_m value will not vary more than ±20 percent from that which would be calculated using a k_m value based on exact animal weight. This is a relativity small variance considering dose separation generally used in deriving the NOAEL, in toxicology studies, which are often twofold separations. For example, suppose a NOAEL in rats is 75 mg/kg and the average rat weight is 250 g. The k_m value for a 250 g rat is 7.0.

$HED = 75 \times (7/37) = 14$ mg/kg in humans.

Using the standard k_m value of 6 for rats,

$HED = 75 \times (6/37) = 12$ mg/kg in humans.

The HED calculated with the standard k_m value of 6 is within 15 percent of the value calculated using the actual k_m value of 7. As shown in Table 3, the body weights producing k_m factors for which the nominal, integer conversion factor was within 20 percent of the calculated factor covered a broad range. This working weight range encompassed the animal weights expected for the majority of studies used to support starting doses in humans.

Table 3: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Ar

Species	Reference Body Weight (kg)	Working Weight Range[a] (kg)	Body Surface Area (m^2)	To Convert Dose in mg/kg to Dose in mg/m^2 Multiply by k_m	To Convert An to HED[b] in Divide Animal Dose B
Human	60	---	1.62	37	---
Child[c]	20	---	0.80	25	---
Mouse	0.020	0.011-0.034	0.007	3	12.3
Hamster	0.080	0.047-0.157	0.016	5	7.4
Rat	0.150	0.080-0.270	0.025	6	6.2
Ferret	0.300	0.160-0.540	0.043	7	5.3
Guinea pig	0.400	0.208-0.700	0.05	8	4.6
Rabbit	1.8	0.9-3.0	0.15	12	3.1
Dog	10	5-17	0.50	20	1.8
Primates:					
Monkeys[d]	3	1.4-4.9	0.25	12	3.1
Marmoset	0.350	0.140-0.720	0.06	6	6.2
Squirrel monkey	0.600	0.290-0.970	0.09	7	5.3
Baboon	12	7-23	0.60	20	1.8
Micro-pig	20	10-33	0.74	27	1.4
Mini-pig	40	25-64	1.14	35	1.1

[a] For animal weights within the specified ranges, the HED for a 60 kg human calculated using the standard k_m value will not vary m from the HED calculated using a k_m value based on the exact animal weight.

[b] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[c] The k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

[d] For example, cynomolgus, rhesus, and stumptail.

For the typical species used in nonclinical safety studies, Table 3 also shows the body surface area in m^2 for an animal at a particular *reference* weight. For example, a 400 g guinea pig has a body surface area of approximately 0.05 m^2. These values come from published sources with surface area determined experimentally by various methods. Compilations of this type of data can be found in published references (Spector 1956).

For animal weights outside the working weight range in Table 3, or for species not included in the table, an alternative method is available for calculating the HED. In these cases the following formula can be used:

$$\text{HED} = \text{Animal dose (mg/kg)} \times [\text{animal weight (kg)} \div \text{human weight (kg)}]^{0.33}$$

For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor. For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor.

The k_m analysis addresses only half of the HED conversion process. The range of human sizes should also be considered to convert the mg/m^2 dose back to an HED dose in mg/kg. To examine the effect of both animal and human weights on the conversion factor, the principle of allometry was used. Interspecies biologic parameters are often related by the power function $Y = aW^b$ where W is body weight and b (allometric exponent) is the slope of the log-log plot, $\log y = b \times \log W + C$. Using algebraic manipulation (see Appendix C), one can derive an equation for converting an animal dose to the HED based on the body weights of the human and the animals for a given allometric exponent. For converting an animal NOAEL in mg/kg to the HED in mg/kg, the equation is:

(ii) HED = animal NOAEL x $(W_{animal}/W_{human})^{(1-b)}$

Since body surface area is believed to scale with an allometric exponent (b) of 0.67, one can explore how the animal and human body weights affect the conversion factor $(W_{animal}/W_{human})^{0.33}$.

The conversion factor was calculated over a range of animal weights and a range of human weights from 50-80 kg. The results are summarized in Table 4. Column B is the weight range of the animals used to calculate, in conjunction with the 50-80 kg range in humans, the conversion factor. The extremes of the conversion factors for the permutations chosen are shown in columns C and D. The proposed standard conversion factors are shown in column E. The percentage difference of these extremes from the standard is shown in column F. Finally, the range of animal weights that produced a conversion factor for a 60 kg human within 20 percent of the standard factor is shown in column G. The ±10 percent and ±20 percent intervals across the entire range of weights are graphically illustrated for rats in Table 5.

Table 4: Effect of Body Weight on Human Equivalent Dose Conversions[a]

A	B	C	D	E	F	G
Species	Animal Weight Range[b] (kg)	Conversion Factor[c]: sm animal lg human	Conversion Factor[c]: lg animal sm human	Conversion Factor[c]: Standard[d]	% Difference of Extreme[e] from Standard	±20% Range[f] for 60 kg Human (kg)
Mouse	0.018-0.033	0.060	0.089	0.081	-22%	0.015-0.051
Rat	0.090-0.400	0.106	0.213	0.162	-35%	0.123-0.420
Rabbit	1.5-3.0	0.269	0.395	0.324	+22%	1.0-3.4
Monkey	1.5-4.0	0.319	0.435	0.324	+34%	1.0-3.4
Dog	6.5-13.0	0.437	0.641	0.541	-19%	4.7-16.2

[a] conversion factor = $(W_{animal}/W_{human})^{0.33}$

[b] human weight range used was 50-80 kg (110-176 lb)

[c] HED in mg/kg equals animal dose in mg/kg multiplied by this value

[d] See Table 1

[e] extreme from column C or D

[f] range of animal weights that produced a calculated conversion factor within 20 percent of the standard factor (column E) when human weight was set at 60 kg

Table 5: Human and Rat Body Weights Producing Body Surface Area Dose Conversion Factors Within 10 Percent and 20 Percent of the Standard Factor (0.162)

EFFECT OF BODY WEIGHT ON BSA-CF							
HED = animal NOAEL· (W_{animal}/W_{human})exp(1-b), b = 0.67 for mg/m^2 conversion							
Standard conversion to mg/kg = 0.162				± 10%	0.146-0.178		
				± 20%	0.130-0.194		
Rat Body Weight (kg)	Human Body Weight (kg)						
	50	55	60	65	70	75	80
0.090	0.124	0.120	0.117	0.114	0.111	0.109	0.106
0.100	0.129	0.125	0.121	0.118	0.115	0.113	0.110
0.110	0.133	0.129	0.125	0.122	0.119	0.116	0.114
0.120	0.137	0.132	0.129	0.125	0.122	0.119	0.117
0.130	0.140	0.136	0.132	0.129	0.126	0.123	0.120
0.140	0.144	0.139	0.135	0.132	0.129	0.126	0.123
0.150	0.147	0.142	0.138	0.135	0.132	0.129	0.126
0.160	0.150	0.146	0.141	0.138	0.134	0.131	0.129
0.170	0.153	0.149	0.144	0.141	0.137	0.134	0.131
0.180	0.156	0.151	0.147	0.143	0.140	0.137	0.134
0.190	0.159	0.154	0.150	0.146	0.142	0.139	0.136
0.200	0.162	0.157	0.152	0.148	0.145	0.141	0.138
0.210	0.164	0.159	0.155	0.151	0.147	0.144	0.141
0.220	0.167	0.162	0.157	0.153	0.149	0.146	0.143
0.230	0.169	0.164	0.159	0.155	0.152	0.148	0.145
0.240	0.172	0.166	0.162	0.157	0.154	0.150	0.147
0.250	0.174	0.169	0.164	0.160	0.156	0.152	0.149
0.260	0.176	0.171	0.166	0.162	0.158	0.154	0.151
0.270	0.179	0.173	0.168	0.164	0.160	0.156	0.153
0.280	0.181	0.175	0.170	0.166	0.162	0.158	0.155
0.290	0.183	0.177	0.172	0.168	0.164	0.160	0.157
0.300	0.185	0.179	0.174	0.179	0.165	0.162	0.158
0.310	0.187	0.181	0.176	0.171	0.167	0.163	0.160
0.320	0.189	0.183	0.178	0.173	0.169	0.165	0.162
0.330	0.191	0.185	0.180	0.175	0.171	0.167	0.163
0.340	0.193	0.187	0.181	0.177	0.172	0.169	0.165
0.350	0.194	0.188	0.183	0.178	0.174	0.170	0.167
0.360	0.196	0.190	0.185	0.180	0.176	0.172	0.168
0.370	0.198	0.192	0.187	0.182	0.177	0.173	0.170
0.380	0.200	0.194	0.188	0.183	0.179	0.175	0.171
0.390	0.202	0.195	0.190	0.185	0.180	0.176	0.173
0.400	0.203	0.197	0.191	0.186	0.182	0.178	0.174
0.410	0.205	0.199	0.193	0.188	0.183	0.179	0.175
0.420	0.207	0.200	0.194	0.189	0.185	0.181	0.177
0.430	0.208	0.202	0.196	0.191	0.186	0.182	0.178
0.440	0.210	0.203	0.197	0.192	0.188	0.183	0.180
0.450	0.211	0.205	0.199	0.194	0.189	0.185	0.181
0.460	0.213	0.206	0.200	0.195	0.190	0.186	0.182

The following are conclusions from these analyses:

- The ±20 percent interval around the standard conversion factor includes a broad range of animal and human weights.

- Given that the human weights will vary broadly, it is not usually necessary to be concerned about the affect of the variation of animal weights within a species on the HED calculation.

- If an extreme animal weight is encountered in a toxicology study, one can calculate an accurate conversion factor using $(W_{animal}/W_{human})^{0.33}$.

APPENDIX C:
Derivation of the Interspecies Scaling Factor $(W_a/W_h)^{(1-b)}$

Power equation $(mg) = aW^b$

$\log(mg) = \log(a) + b \bullet \log(W) = b \bullet \log(W) + c$

Given the weights of animal and human, and animal dose in mg/kg, solve for HED in mg/kg:

Let H = mg/kg dose in humans
A = mg/kg dose in animals
W_b = weight of human
W_a = weight of animal

for animal $\log(mg) = \log(a) + b \bullet \log(W_a) = b \bullet \log(W_a) + c$

replace mg $\log(A \bullet W_a) = b \bullet \log(W_a) + c$

solve for c $c = \log(A \bullet W_a) - b \bullet \log(W_a)$

$= \log(A) + \log(W_a) - b \bullet \log(W_a)$

$= \log(A) + (1-b)\log(W_a)$

likewise for human $c = \log(H) + (1-b)\log(W_h)$

equate two equations $\log(A) + (1-b)\log(W_a) = \log(H) + (1-b)\log(W_h)$

solve for log(H) $\log(H) = \log(A) + (1-b)\log(W_a) - (1-b)\log(W_h)$

$= \log(A) + (1-b)[\log(W_a) - \log(W_h)]$

$= \log(A) + \log[(W_a/W_h)^{(1-b)}]$

$\log(H) = \log[A \bullet (W_a/W_h)^{(1-b)}]$

solve for H $H = A \bullet (W_a/W_h)^{(1-b)}$

For example, using mg/m^2 normalization (b = 0.67) the predicted human MTD in mg/kg based on a rat LD_{10} in mg/kg is $MTD = LD_{10} \bullet (W_a/W_h)^{0.33}$.

Likewise the HED in mg/kg based on a surface area conversion given an animal NOAEL is $HED = NOAEL \bullet (W_a/W_h)^{0.33}$.

APPENDIX D:
Examples of Calculations for Converting Animal Doses to Human Equivalent Doses

This appendix provides examples of specific calculations to be taken in deriving an HED based on standardized factors.

Tables 1 and 3 provide standardized conversion factors for changing animal or human doses expressed as mg/kg to doses expressed as mg/m^2. Tables 1 and 3 also have factors (and divisors) for converting animal doses in mg/kg to the human dose in mg/kg that is equivalent to the animal dose if both were expressed on a mg/m^2 basis. This human dose in mg/kg is referred to as the HED.

Example 1: Converting to mg/m^2 HED

To convert an animal or human dose from mg/kg to mg/m^2, the dose in mg/kg is multiplied by the conversion factor indicated as k_m (for mass constant). The k_m factor has units of kg/m^2; it is equal to the body weight in kg divided by the surface area in m^2.

formula:	$mg/kg \times k_m = mg/m^2$
to convert a dose of 30 mg/kg in a dog:	$30 \times 20 = 600\ mg/m^2$
to convert a dose of 2.5 mg/kg in a human:	$2.5 \times 37 = 92.5\ mg/m^2$

Example 2: Converting to mg/kg HED in two steps

To calculate the HED for a particular dose in animals, one can calculate the animal dose in mg/m^2 by ***multiplying*** the dose in mg/kg by the k_m factor for that species as described in Example 1. The dose can then be converted back to mg/kg in humans by ***dividing*** the dose in mg/m^2 by the k_m factor for humans.

formula: (animal mg/kg dose × animal k_m) ÷ human k_m = human mg/kg dose
to calculate the HED for a 15 mg/kg dose in dogs:
$(15 \times 20) \div 37 = 300\ mg/m^2 \div 37 = 8\ mg/kg$

Example 3: Converting to mg/kg HED in one step

The calculation in Example 2 can be simplified by combining the two steps. The HED can be calculated directly from the animal dose by ***dividing*** the animal dose by the ratio of the human/animal k_m factor (third column in Table 1) or by ***multiplying*** by the ratio of the animal/human k_m factor (fourth column in Table 1).

Division method

NOAEL	calculation	HED
	mg/kg ÷ [$k_{mhuman}/k_{manimal}$]	
15 mg/kg in dogs	15 mg/kg ÷ 1.8 =	8 mg/kg
50 mg/kg in rats	50 mg/kg ÷ 6.2 =	8 mg/kg
50 mg/kg in monkeys	50 mg/kg ÷ 3.1 =	16 mg/kg

Multiplication method

NOAEL	calculation	HED
	mg/kg × [$k_{manimal}/k_{mhuman}$]	
15 mg/kg in dogs	15 mg/kg × 0.541 =	8 mg/kg
50 mg/kg in rats	50 mg/kg × 0.162 =	8 mg/kg
50 mg/kg in monkeys	50 mg/kg × 0.324 =	16 mg/kg

APPENDIX E:
Selection of Maximum Recommended Starting Dose for Drugs Administered Systemically to Normal Volunteers